

REMARKS

Claims 27, 29-42, 44-47, and 49-55 are pending in the present application. Reconsideration is respectfully requested in view of the following comments and the amendments above.

The rejection of Claims 27-29, 35, 39, 43, 44, and 48 under 35 U.S.C. §112, first paragraph (written description; new matter), is obviated by the present amendment.

Applicants make no statement in regard to the propriety of this ground of rejection and in no way acquiesce to the same. However, in order to expedite examination of this application, Applicants have deleted the phrase “all or part of” from Claim 27. Therefore, this ground of rejection is now believed to be moot.

Withdrawal of this ground of rejection is requested.

The rejection of Claims 27-29, 35, 39, 43, 44, and 48 under 35 U.S.C. §112, first paragraph (written description and enablement), is obviated by the present amendment.

In regard to the Examiner’s criticisms of the claims in relation to SEQ ID NO: 11, Applicants note that this issue is now moot in view of the deletion of this sequence by amendment herein.

Turning to the claims as currently presented, Applicants wish to offer the following remarks. In particular, to address the Examiner’s assertion that “extensive random unpredictable experimentation would be required to determine useable peptide molecules for a given purpose,” Applicants offer the following:

MPEP §2164.04 states:

A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the

subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

Applicants submit that the present specification provides ample description and support for the skilled artisan to produce polypeptide molecules containing at least 50 consecutive amino acids of SEQ ID NO:3, since the amino acid sequence of SEQ ID NO: 3 is provided in the present specification. Further, the skilled artisan may, without undue experimentation, easily synthesize at least 50 consecutive amino acids according to the methods described at least on page 11 of the specification; i.e., by the methods of Houbert-Weyl or R.D. Merrifield. These methods have been known in the art for at least 31 years!

Following synthesis, the particular peptides can be tested for their ability to confer immunity against malaria as described throughout the Examples in the present specification. More particularly, the skilled artisan would initially, after synthesis, try to test the peptides in mice as illustrated in Example 6 of the specification. After initial screening, the peptides can be further tested in chimpanzees as exemplified in Examples 2 (2.1 to 2.5), 4 (4.1 to 4.4) and 7. Thus, there are numerous working examples in the present specification that would guide the person skilled in the art to test for immunogenicity.

Furthermore, the specification provides additional guidance with respect to the particular peptides that should be chosen. For example, the specification describes that B and T responses are important and have been identified in the non repeat proteins of the 5' and 3' regions of LSA-3 (page 19). At least Example 3 of the specification describes ways to identify CTL epitopes and that observed protection that depended on cellular responses is important for a vaccine.

Moreover, on page 35 the specification describes that inhibitory activity is displayed in the development of *Plasmodium falciparum* with interferons. Thus, the person skilled in the art can also test to determine whether the peptides have this type of activity.

Thus, Applicants submit that the specification provides enough guidance to permit the skilled artisan to obtain and test the polypeptide molecules containing at least 50 consecutive amino acids of SEQ ID NO: 3 without undue experimentation and, as such, satisfy the requirements of MPEP §2164.04.

Applicants caution that the quantity and complexity of testing should not be a criteria to render a rejection under enablement. As set forth in *Massachusetts Institute of Technology v. AB Fortia*, 774 F. 2d 1104, 227 USPQ 428 (Fed. Cir. 1985):

[T]he fact that experimentation may be complex...does not necessarily make it undue, if the art typically engages in such experimentation.

In fact, MPEP §2164.06 states:

... quantity of experimentation needed to be performed by one skilled in the art is only one factor involved in determining whether "undue experimentation" is required to make and use the invention. "[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." In re Colianni, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977). "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

Applicants submit that, with the present specification in hand, determination of polynucleotide sequences that fall within the scope of the present invention would require nothing more than routine experimentation, even though the protocols may appear to be complex. As such, Applicants submit that the claims of the present application are fully enabled within the context of 35 U.S.C. §112, first paragraph.

Based on the foregoing, Applicants submit that the present claims are fully described and enabled by the specification, especially when viewed in conjunction with the common

knowledge available in the art. As such, withdrawal of these grounds of rejection is requested.

The rejection of Claims 27-29, 35, 39, 43, 44 and 48 under 35 U.S.C. §112, second paragraph, is obviated by amendment.

Applicants have amended the claims to be free of the Examiner's criticisms. More specifically, Claim 27 has been amended to recite at least 50 amino acids of SEQ ID NO: 3. It should be clear from the language in this claim, that any polypeptide molecule having one or more polypeptides of SEQ ID NOS: 10, 12, 13, 14 and 15 are *in fact* excluded from the claim language. Since "all or part" has been deleted from this claim, it is clear that peptides shorter than at least 50 consecutive amino acids are not encompassed by the claim. Moreover, Claim 35 has been amended to ensure proper antecedent basis.

In view of the amendments herein, withdrawal of this rejection is respectfully requested.

The rejection of Claims 27-29 under 35 U.S.C. § 102 (a) over Barnes et al is traversed.

The Examiner asserts that the argument filed on September 3, 2004, has been considered but is not persuasive. The Examiner further asserts, "Applicant cannot rely upon the foreign priority claim to overcome this rejection because the requirements of 35 U.S.C. §119 have not been met in... the instant application. The certified copy of the priority document is absent."

Applicants submit that this assertion by the Examiner is incorrect in view of the record of the present application. On December 22, 2000, Applicants filed a Request for Priority (copy **submitted herewith**), in which Applicants claimed priority to FR9507007.

Further, in the Request for Priority, Applicants noted that a certified copy of the same was submitted to the International Bureau in PCT application PCT/FR96/00894. It was further noted that receipt of the certified copy by the International Bureau in a timely manner under PCT Rule 17.1(a) was acknowledged as evidenced by PCT/IB/304 (copy **submitted herewith**).

Applicants again note that Barnes et al was published in August 1995, whereas this application claims priority to French application 95/07007 filed on June 13, 1995. Since the certified copy of FR9507007 was timely submitted to the International Bureau in PCT application PCT/FR96/00894, a certified English translation of this French application was filed in the parent application (US 08/973,462), and a certified English translation of this French application was again filed with the response on September 3, 2004 (copy of the date-stamped filing receipt is **submitted herewith**), the Examiner's rejection is not proper. Nonetheless, in order to ensure that no further errors occur in this regard, Applicants **enclose herewith another** copy of the certified English translation of the French priority document (FR9507007). Accordingly, Applicants submit that Barnes et al should not be prior art and this ground of rejection should be withdrawn.

Acknowledgment of withdrawal of this rejection is respectfully requested.

The rejection of Claims 27-29, 35, 39 and 43 under 35 U.S.C. § 102 (f), is respectfully traversed.

In rendering this rejection, the Examiner deems that since U.S. Patent Nos. 6,319,502 B1 and 6,270,771 have different inventors than the presently claimed invention and describe similar subject matter, the presently claimed inventors did not contribute to the above-captioned invention. Applicants disagree with the Examiner's conclusions for the reasons already of record.

It should be noted that Pierre Druihle is a common inventor for both the U.S. issued patents and the present invention. Furthermore, U.S. Patent Nos. 6,270,771 B1 and 6,319,502 do not disclose the entire LSA-3 sequence, which is the subject of the present application. Moreover, the partial LSA-3 sequence disclosed in these issued patents is furthermore excluded from the claims.

To further evidence that the present invention was not derived from Ms. Claudine Guerin-Marchand, Applicants provide the **enclosed** Rule 132 Declarations executed independently by Pierre Druilhe (the Druilhe Declaration) and Pierre Daubersies (the Daubersies Declaration). In the Druilhe Declaration, the history of the joint invention of Ms. Claudine Guerin-Marchand and Mr Pierre Druilhe appearing in U.S. Patent Nos. 6,319,502 B1 and 6,270,771 is described. Also in the Druilhe Declaration, Mr. Pierre Druilhe describes the role of the inventors of the present application. Further, in the Daubersies Declaration, Mr. Pierre Daubersies describes his role in the invention claimed in the present application. Therefore, Applicants submit that the presently claimed invention was not derived from Ms. Claudine Guerin-Marchand.

In view of the foregoing, withdrawal of this rejection is respectfully requested.

The rejections of (a) Claims 27-29, 35, 39 and 43 under 35 U.S.C. § 102 (b) over Guerin-Marchand et al (WO92/13884) in the light of the translation provided in U.S. Patent No. 6,270,771, and (b) Claims 27-29, 35, 39 and 43 under 35 U.S.C. § 102 (e) over Guerin-Marchand (U.S. Patent No. 6,319,502) are respectfully traversed.

U.S. Patent No. 6,270,771 discloses various sequences from LSA-1, which have the following repetitive sequence:

X₁DLEQX₂RX₃AKEKLQX₄QQQ

where X₁ is Ser or Arg

X₂ is Glu or Asp

X₃ is Arg or Leu

X₄ is Glu or Gly.

This sequence is not present in SEQ ID NO: 3 of the present invention. Thus, even sequences which are longer that include the above sequence are not claimed in the present invention.

The other sequences described in U.S. Patent No. 6,270,771 are derived from the clone DG729S. The 729Sprotein is described at column 5 as SEQ ID No:24 and has 151 amino acids. As pointed out by the Examiner, SEQ ID NO: 11 of the present invention is not disclosed in SEQ ID NO: 3. The remaining sequence of SEQ ID NO: 24 in the prior art is excluded from the presently claimed invention as SEQ ID NO: 10.

The remaining sequences in U.S. Patent 6,270,771, which are described are SEQ ID NO: 25, which is the equivalent sequence to excluded SEQ ID NO: 12 of the present invention; SEQ ID NO:26, which is the equivalent excluded sequence of SEQ ID NO: 13 of the presently claimed invention; SEQ ID NO: 27, which is the equivalent to the excluded SEQ ID NO: 14 of the presently claimed invention; and SEQ ID NO: 28, which is the equivalent to the excluded SEQ ID NO: 15 of the presently claimed invention.

Furthermore, SEQ ID NOS: 25 to 28 have amino acid sequences that are less than 50 consecutive amino acids. As pointed out by the Examiner, U.S. Patent 6,270,771 discloses that the invention encompasses "any sequence comprising 4 to 5 amino acids up to the maximal number of the sequences described above." Since, SEQ ID NOs. 25 to 28 have 47, 26, 27 and 27 total amino acids, these sequences cannot be said to encompass a polypeptide molecule having at least 50 consecutive amino acids of SEQ ID NO: 3 of the present invention.

Therefore, U.S. Patent No. 6,270, 771 fails to disclose or suggest the presently claimed invention. In order for a reference to anticipate an invention, the reference “must teach every element of the claim” (MPEP §2131). Accordingly, U.S. Patent No. 6,270, 771 does not anticipate the invention as presently claimed.

For the same reasons as stated above, the presently claimed invention is not anticipated by U.S. Patent 6,319, 502, which is a divisional of U.S. 6,270,771. Since both issued patents have the same specification, U.S. Patent 6,319, 502 does not anticipate the claimed invention. In summary U.S. Patent 6,319,502 teaches sequences that are not presently being claimed, are excluded from the claims or are not of sufficient length to anticipate the presently claimed invention.

Therefore, in view of the above, withdrawal of these rejections is respectfully requested.

Finally, Applicants remind the Examiner that MPEP §821.04 states:

...if applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims which depend from or otherwise include all the limitations of the allowable product claim *will be rejoined*. (emphasis added)

Accordingly, should the elected invention be found allowable, Applicants request that withdrawn process claims be rejoined and examined.

Application No. 09/742,096
Reply to Office Action of December 3, 2004

Applicants submit that the application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.
Norman F. Oblon



Vincent K. Shier, Ph.D.
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(OSMMN 08/03)



PATENT

Attorney Docket No.200773USODiv

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)

Pierre DRUILHE et al)

) Group Art Unit: 1641

Serial No.:09/742,096)

) Examiner: James Leslie GRUN

Filed: December 22, 2000)

For: MALARIAL PRE-ERYTHROCYTIC STAGE POLYPEPTIDE MOLECULES

1.132 Declaration

Commissioner for Patents.

P.O. Box 1450

Alexandria, Virginia 22313-1450

I, Pierre Daubersies do hereby declare the following:

- (1) I Am currently working in the Research and Development Department at Merck LIPHA SANTE in France. Prior to being employed by Merck, I worked in the Laboratory of Parasitology at Institut Pasteur under the head of the Department, Mr. Pierre Druilhe. During the years of 1992 to 1994, I was a post-doctoral Fellow in Mr. Druihle's laboratory working on a

project to clone, sequence and characterize LSA-3. My *Curriculum Vitae* is enclosed. I am one of the inventors of the above-captioned U.S. patent application.

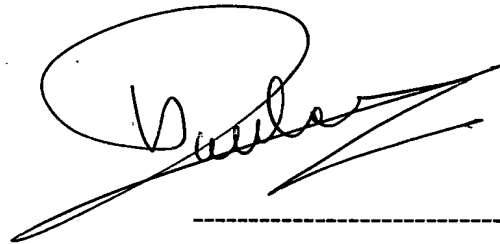
(2) In the present invention the entire *lsa-3* gene was cloned and sequenced, which was the goal of my post-doctorate studies.. To clone and sequence the entire *lsa-3* gene was in fact quite difficult since the genome of *Plasmodium falciparum* is very rich in A: T bases (80%), thus there is a rarity of restriction sites which can be used and also instability of cloning certain fragments when they are inserted into plasmid vectors. Thus, it took further ingenuity to obtain full length LSA-3, analyze its sequence, as well as to determine whether there was any polymorphism in different isolates, which is generally the case between different clones, strains and isolates of *Plasmodium*, as well as to determine the antigenicity in chimpanzees of LSA-3 and fragments thereof.

(3) Secondly, I am one of the true inventors of the claimed subject matter of the present patent application and did not derive the claimed subject matter from Ms. Claudine Guerin-Marchand, who at that epoch was working in the same laboratory. Although Ms. Claudine Guerin-Marchand did obtain a clone 729S while working under the supervision of Professor Druilhe, this was only a 151 amino acid antigen of LSA-3, which is a short fragment.

(4) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

13-03-05

Date

A handwritten signature in black ink, appearing to read 'Daubersies', is written over a horizontal dashed line. The signature is stylized with a large loop at the beginning and a long, sweeping stroke extending to the right.

Pierre Daubersies

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
)
 Pierre DRUILHE et al)
) Group Art Unit: 1641
 Serial No.:09/742,096)
) Examiner: James Leslie GRUN
 Filed: December 22, 2000)
)
 For: MALARIAL PRE-ERYTHROCYTIC STAGE POLYPEPTIDE MOLECULES

1.132 Declaration

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

I, Pierre Druilhe do hereby declare the following:

- (1) I am currently the director of the Biomedical Parasitology Unit at Institut Pasteur in Paris, France and have been the director of this department for many years. As can be seen from my attached *Curriculum Vitae*, I have published 260 articles and have 10 U.S. Patents issued in my name. I am one of the inventors of the above-captioned U.S. patent application.

- (2) The primary scientific interest in my laboratory is focused on the understanding of *Plasmodium falciparum*/human immune reactions, both at the level of the pre-erythrocytic stages (sporozoite and liver stage), as well as the merozoite stage. The main activities with these scientific studies in my laboratory are aimed at vaccines to treat or prevent malaria, as well as drug discovery in this area.
- (3) As head of a laboratory at Institut Pasteur, I supervise and am responsible for many researchers, scientific trainees, as well as other personnel such as laboratory technicians and office staff. My laboratory also collaborates on particular scientific projects with various scientific groups not associated with Institut Pasteur.
- (4) I have read and understood the last U.S. Official Action dated December 3, 2004. As I understand, the Examiner has rejected claims 27 to 29, 35, 39 and 43 on the basis that Mr. Pierre Daubersies and I did not invent the subject matter which is currently claimed and sought to be patented. It is my further understanding that the rationale behind this rejection is that issued U.S. Patents 6,270,771 B1 and 6,319,502 B1 have myself and Claudine Guerin-Marchand as inventors, while in the above present patent application the other inventor is Pierre Daubersies. Since a partial sequence of a 729S protein was disclosed in issued patents 6,270,771 and 6,319,502, the Examiner maintains that the present inventorship is not correct. I disagree with the Examiner's conclusions for the following reasons.
- (5) U.S. Patent Nos. 6,270,771 and 6,319,502 are based on the discovery and sequencing of peptides from LSA-1 (liver stage antigen 1). At that epoch and as described in those patent specifications, one clone that was derived from our extensive search of the genomic library of *Plasmodium falciparum* was directed to a protein different from LSA-1; i.e., LSA-3 clone DG729S. The sequencing of this clone revealed a polypeptide of 151 amino acids, which is only a partial sequence of the LSA-3 protein. This scientific work was in fact the conception of myself

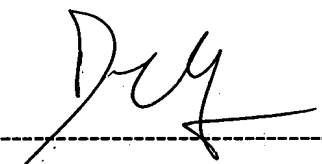
and Ms. Claudine Guerin-Marchand, who was a researcher in my laboratory at that time. After appropriate evaluation by Institut Pasteur's Patent and Invention Office, it was decided that Claude Guerin-Marchand and myself were inventors of at least one claim for U.S. Patent 6,270,771 and 6,319,502.

- (6) In contrast, in the present invention the entire *lsa-3* gene was cloned and sequenced. To clone and sequence the entire *lsa-3* gene was in fact quite difficult since the genome of *Plasmodium falciparum* is very rich in A: T bases (80%), thus there is a rarity of restriction sites which can be used and also instability of cloning certain fragments when they are inserted into plasmid vectors. Thus, it took further ingenuity to obtain full length LSA-3, analyze its sequence, as well as to determine whether there was any polymorphism in different isolates, which is generally the case between different clones, strains and isolates of *Plasmodium*, as well as to determine the antigenicity in chimpanzees of LSA-3 and fragments thereof.
- (7) In this respect, a post-doctoral fellow, Mr. Pierre Daubersies undertook the project to clone LSA-3 with myself. After appropriate evaluation by Institut Pasteur's Patent and Invention Office, it was concluded that myself and Mr. Duabersies were considered inventors for at least one claim in the present patent application.
- (8) I am one of the true inventors of the claimed subject matter of the present patent application and did not derive the invention from Ms. Claudine Guerin-Marchand.

(9) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

7-03-2005

Date


Pierre Druilhe



OSMM&N No. 200773US0DIV

Dept.: CHEMICAL

By: NFO:JK:jot

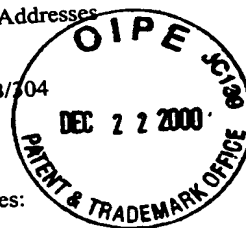
Serial No. NEW APPLICATION

In the matter of the Application of: Pierre DRUILHE et al.

For: MALARIAL PRE-ERYTHROCYTIC STAGE POLYPEPTIDE MOLECULES

The following has been received in the U.S. Patent Office on the date stamped hereon:

- ☒ 41 pp. Specification & 26 Claims/Drawings 26 Sheets
- ☒ Copy of Combined Declaration, Petition & Power of Attorney 3 pages (executed)
- ☐ List of Inventor Names and Addresses
- ☒ Utility Patent Application
- ☒ Notice of Priority w/PCT/IB/304
- ☒ Check for \$854.00
- ☒ Fee Transmittal Form
- ☐ Assignment/PTO 1595 pages:
- ☐ Letter to Official Draftsman
- ☐ Letter Requesting Approval of Drawing Changes
- ☐ Drawings sheets ☐ Formal
- ☐ Letter
- ☒ Preliminary Amendment
- ☒ Information Disclosure Statement
- ☒ Cited References (6)
- ☒ International Search Report
- ☐ Statement of Relevancy
- ☐ IDS/Related/List of Related Cases
- ☐ Restriction Response
- ☐ Rule 132 Declaration
- ☐ Petition for Extension of Time
- ☐ Notice of Appeal
- ☐ Brief
- ☐ Issue Fee Transmittal
- ☒ White Advance Serial Number Card
- ☐ Small Entity Status is Claimed
- ☒ Substitute Sequence Listing w/Attached Sequence Alignment
- ☐



- ☐ CPA
- ☐ Priority Doc
- ☒ Dep. Acct. Order Form

COPY

☒ PTO-1449

- ☐ Cited Pending Applications
- ☐ Election Response

Due Date: December 26, 2000

OSMM&N File No. 200773US0DIV

Serial No. 09/742,096

In the matter of the Application of: Pierre DRUILHE, et al.

For: MALARIAL PRE-ERYTHROCYTIC STAGE POLYPEPTIDE MOLECULES

Dept.: Chemical

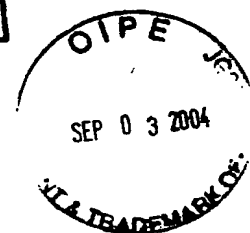
By: NFO/DJP/km

Due Date: September 3, 2004

The following has been received in the U.S. Patent Office on the date stamped hereon

- ☐ Credit Card Form for \$950.00
- ☐ Dep. Acct. Order Form
- ☐ PTO Cover Letter
- ☐ Amendment and Request for Reconsideration w/Annex I attached
- ☐ Petition for Extension of Time (Three-Months)
- ☐ Sequence Listing (Paper)
- ☐ Computer-Readable Sequence Listing (Diskette)
- ☐ Certified English Translation of French Application No. 9507007
- ☐ Letter Submitting Replacement Drawing Sheets for FIGS. 1a-1f, 2a-21, 3a-3d
4b, 5, 6, 7, 8, 9a-9c, 10a-10b (attached)

COPY



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Pierre DRUILHE et al.

GAU:

SERIAL NO: NEW APPLICATION

EXAMINER:

FILED: P E HEREWITH

FOR: MALARIAL PRE-ERYTHROCYTIC STAGE POLYPEPTIDE MOLECULES

REQUEST FOR PRIORITY

ASSISTANT COMMISSIONER FOR PATENTS

WASHINGTON, D.C. 20231

SIR:

- ☒ Full benefit of the filing date of U.S. Application Serial Number 08/973,462, filed February 6, 1998, is claimed pursuant to the provisions of 35 U.S.C. §120.
- ☐ Full benefit of the filing date of U.S. Provisional Application Serial Number , filed , is claimed pursuant to the provisions of 35 U.S.C. §119(e).
- ☒ Applicants claim any right to priority from any earlier filed applications to which they may be entitled pursuant to the provisions of 35 U.S.C. §119 and §120, as noted below.

In the matter of the above-identified application for patent, notice is hereby given that the applicants claim as priority:

<u>COUNTRY</u>	<u>APPLICATION NUMBER</u>	<u>MONTH/DAY/YEAR</u>
France	95/07007	June 13, 1995
WIPO	PCT/FR96/00894	June 12, 1996

Certified copies of the corresponding Convention Application(s)

- ☐ are submitted herewith
- ☐ will be submitted prior to payment of the Final Fee
- ☐ were filed in prior application Serial No. filed
- ☒ were submitted to the International Bureau in PCT Application Number PCT/FR96/00894. Receipt of the certified copies by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.
- ☐ (A) Application Serial No.(s) were filed in prior application Serial No. filed ; and
(B) Application Serial No.(s)
 - ☐ are submitted herewith
 - ☐ will be submitted prior to payment of the Final Fee

Respectfully Submitted,

OBLON, SHYAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Registration No. 24,618

James J. Kelly, Ph.D.
Registration No. 41,504



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TRAITÉ DE COOPERATION EN MATIÈRE DE BREVETS

PCT

NOTIFICATION RELATIVE A LA PRESENTATION DU DOCUMENT DE PRIORITE

(instruction administrative 411 du PCT)

Expéditeur : le BUREAU INTERNATIONAL

Destinataire:


GUTMANN, Ernest
Yves Plasseraud S.A.
3, rue Chauveau-Lagarde
F-75008 Paris
FRANCE

Date d'expédition (jour/mois/année) 24 juillet 1996 (24.07.96)		
Référence du dossier du déposant ou du mandataire B2775A-FL		NOTIFICATION IMPORTANTE
Demande internationale no PCT/FR96/00894	Date du dépôt international 12 juin 1996 (12.06.96)	Date de priorité 13 juin 1995 (13.06.95)
Déposant INSTITUT PASTEUR etc		

La date de réception par le Bureau international du ou des documents de priorité correspondant à la ou aux demandes suivantes est notifiée au déposant:

<u>Demande antérieure no:</u>	<u>Date de priorité:</u>	<u>Pays dans lequel ou pour lequel la demande a été déposée:</u>	<u>Date de réception du document de priorité</u>
95/07007	13 jui 1995 (13.06.95)	FR	23 jui 1996 (23.07.96)

COPY

Bureau international de l'OMPI 34, chemin des Colombettes 1211 Genève 20, Suisse no de télécopieur: (41-22) 740.14.35	Fonctionnaire autorisé: G. Bähr  no de téléphone: (41-22) 730.91.11
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COPY

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below:

That I am knowledgeable in the English language and in the language in which the below identified French Application was filed, and that I believe the English translation of the French Application N° 9507007 filed on 13/06/95 in the name of Institut Pasteur


is a true and complete translation of the above identified French Application as filed.

Date:28/04/2000.....

Full name of the translator

Véronique MARCADÉ

Signature of the translator



Post Office Address

3, rue Chauveau-Lagarde
F-75008 Paris
FRANCE

5

10 POLYPEPTIDE MOLECULES OF THE PRE-ERYTHROCYTIC STAGE OF
MALARIA

15 The parasites responsible for malaria in man display different morphologies in the human host and express different antigens depending on their location in the body. The morphological and antigenic differences of these parasites during their life cycles in man enable different stages of development in the liver and in the blood to be defined: the sporozoite, 20 the infectious form injected by the vector mosquito, transforms rapidly into a schizont in the host's hepatocytes and thereafter infects the erythrocytes. The intrahepatic localization of P.falciparum manifests itself in the expression of a group of antigens 25 specific to this stage of development and which are highly immunogenic under the natural conditions of exposure to the disease. This clinically silent phase is at present the only one against which a very strong, sterilizing immunity can be induced experimentally in 30 man, by injecting irradiated sporozoites capable of entering the hepatocyte and of developing therein but without being able to lead on to the blood stage of the disease. Accordingly, the inventors have concentrated the bulk of their efforts on these two pre-erythrocytic 35 stages. However, these stages are also the most intricate ones to study, and hence the least understood, since it is difficult or even impossible to obtain biological material, the only in vitro study

model affords a very low yield and the best animal model remains the chimpanzee, the use of which is limited and expensive.

5 In order to gain access to the antigens of the pre-erythrocytic stages, the inventors used sera of individuals who had resided for 25 years in a region where the disease is endemic but who were on permanent prophylaxis with chloroquine. These individuals were regularly subjected to infected mosquito bites but did
10 not develop any complete blood infection. Their serum hence contained antibodies directed essentially against the pre-erythrocytic stages, which was verified by immunofluorescence (IF) and western blotting on the 3 stages of the parasite.

15 The use of these sera for screening a library of genomic DNA of the parasitic clone of P.falciparum, the library being constructed in expression vectors in a phage lambda gt11 (V. Rosario, Science 212, 1981, pp. 1037-1038; and Thaithong et al., Transactions of
20 Royal Society of Tropical Medicine and Hygiene, 1984, 78:242-245), led to the demonstration of polypeptides of the pre-erythrocytic stage, in particular the SALSA (sporozoite liver stage antigen) polypeptides described in EP A-0,407,230 and LSA-1 (liver stage antigen)
25 described in WO 92/13884. The present invention relates to new polypeptide molecules specific to the pre-erythrocytic stage, and to their use as active principle of antimalarial vaccine or in methods of diagnosis of the disease.

30 The invention is the outcome of the demonstration by the inventors of the special properties of a particular antigen referred to LSA-3 and of its fragments, which are seen to be candidates with a strong potential for producing an antimalarial
35 vaccine, for the following reasons:

a) when a fraction of LSA-3 was used in combination with another antigen of the same stage of development of the parasite, such as LSA-1, to immunize

chimpanzees, the animal responding to both molecules or only to LSA-3 displays the feature of not having parasites in the blood, of having a substantial decrease of the parasites in the liver and of manifest-
5 ing a substantial recruitment of mononuclear cells indicating a response in terms of cellular immunity;
b) in regions where the disease is endemic, a very clear correlation is observed between the protection of individuals against natural infection by sporozoites
10 and their responses in terms of antibodies against LSA-3;
c) in eight human volunteers immunized by injection of irradiated sporozoites, antibodies directed against LSA-3 are found in each of the four individuals
15 resisting sporozoite infection and in none of the other four volunteers who developed a blood infection;
d) antibodies obtained against the peptide DG729 in WO 92/13884, already described, give a cross-reaction with the sporozoite and liver stages of the murine parasite P.yoelii, which permits a significant
20 exploitation of the mouse model. In vitro, the human antibodies immunopurified on DG729 are capable, even at very low concentrations, of blocking the entry of P.yoelii sporozoites into mouse hepatocytes. In vivo,
25 mice immunized with DG729 are fully or partially protected against infection by P.yoelii sporozoites;
e) lastly, some epitopes, in particular in the non-repeat portions of the molecule, stimulate the secretion of interferon- γ by monocytes, this mediator
30 enabling the intrahepatic development of the parasite to be inhibited (S. Mellouk et al., The Jour. Of Immun. 139, 4192-4195, 1987);

All these properties, some of which will be demonstrated in detail in the experiments described
35 later, show that the LSA-3 antigen displays both good antigenicity and good immunogenicity.

The inventors were able to confirm and define the specificity of the stages of expression of the

molecule; in the sporozoites, this expression was confirmed by the surface immunofluorescence of several strains and isolates. In Western Blot analysis, the LSA-3 molecule appears as a protein of molecular weight 200,000 daltons. While the messenger RNAs of sporozoites could not be obtained in sufficient amounts for a northern blot analysis, reverse PCR experiments confirmed the expression of LSA-3 at this stage. In infected hepatocytes, LSA-3 is observed in the parasitophorous vacuole of the parasite by immunofluorescence using antibodies against the repeat and non-repeat regions of the protein, as well as by electron microscopy.

A fragment of LSA-3 designated 729S, as well as three peptides designated NRI and NRII included in the non-repeat portion and 729R included in the repeat portion, have been described in Application WO 92/13884. Nevertheless, this document does not mention the special properties mentioned above, or other fragments of LSA-3 which could be either longer or shorter, included or combined with these fragments, which might display especially advantageous properties for use in vaccines.

The subject of the invention is polypeptide molecules containing at least ten consecutive amino acids of the amino acid sequence shown in Figure 2 and designated SEQ ID No. 2, and representing LSA-3, the following polypeptides being excluded:

- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQEQQHNVEE
- 30 - VEESVEENDEESVEENVEENVENNDDGSVASSVEESIASSVDESIDSSIE-
ENVAPTVEEIVAPTVEEIVAPSVVEKCAPSVVEESVAPSVVEESVAEMLKER
(729S)
- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQEQQHN
- DELFNELLNSVDVNGEVKENILEESQ, (NRI)
- 35 - LEESQVNDDIFNSLVKSVQEQQHNV, (NRII)
- VESVAPSVVEESVAPSVVEESVAENVEESV. (729RE)

Other molecules according to the invention contain at least 20 consecutive amino acids or at least 50.

5 This set of polypeptides and the LSA-3 molecule are, throughout hereinafter, "polypeptides of the invention".

The experimental results and the comparisons of sequences between different P.falciparum isolates indicate the existence of at least 70% homology between
10 equivalent antigens of the liver stage of the parasite. Thus any peptide molecule displaying at least 70% homology with any one of the molecules defined above forms part of the invention, as do those displaying at least 70% homology with the following sequence:

15 Leu Leu Ser Asn Ile Glu Glu Pro Lys Glu Asn Ile Ile Asp
Asn Leu Leu Asn Asn Ile (CT1)

lying between amino acids 140 and 159 of K1 or 23 and 42 of T9/96.

Likewise forming part of the invention are the polypeptide molecules displaying at least 70% homology with
20 the sequence depicted in Figure 3, which depicts a portion of LSA-3 in T9/96: the DNA of this P.falciparum isolate was digested with restriction enzymes, then cloned into lambda gt11 and thus enabled the gene
25 library of this isolate, already described above, to be constituted.

Conjugates consisting of a polypeptide originating from LSA-3 linked covalently via a lysine bridge to saturated or unsaturated lipid residues also
30 form part of the invention, more especially when the lipid residue is a palmitoyl or a palmityl or an oleyl. C₁₆ or C₁₈ residues were thus coupled via a lysine bridge to the peptides NRI, NRII, 729RE and CT1 already depicted above. The method of synthesis used for these
35 conjugates is described in Bourgault, Journal of Immunology, 149, 3416 (1992) and Rouaix, Vaccine, 12, 1209 (1994).

The invention also covers immunogenic compositions containing at least one polypeptide molecule or one conjugate described above, as well as the vaccines containing these immunogenic compositions. Other immunogenic epitopes, in particular LSA-1, SALSA, STARP, have already been described in EP A-0407230 and in WO 92/13884. The vaccine compositions according to the invention can advantageously contain a mixture of immunogenic peptides originating from LSA-3 and of the peptides or antigens originating from LSA-1, SALSA or STARP; a more especially advantageous mixture could be the one consisting, on the one hand of NRI, NRII or whole LSA-3, these being coupled or otherwise to a lipid residue, and on the other hand the peptides SALSA-1, SALSA-2 or the SALSA antigen coupled or otherwise to a lipid residue.

All polypeptide molecules corresponding to the above definition and displaying at least 70% homology with the polypeptides LSA-3, CT1, NRI, NRII or 729RE may be combined in homologous or heterologous fashion with other peptide sequences or sequences originating from another antigen of the different stages of P.falciparum.

The invention also covers the polyclonal or monoclonal antibodies which specifically recognize the polypeptide molecules of the invention.

These molecules of the invention may be used for carrying out diagnostic methods and producing kits enabling the existence of P.falciparum infection to be detected; this method can be either an assay of circulating specific antibodies, by carrying out standard serological methods by bringing one of the above antigens into contact with a biological fluid of the individual in question, or methods of assay of antigens using polyclonal or monoclonal antibodies obtained by standard methods for obtaining such antibodies with the corresponding antigens. In the diagnostic outfits or kits of the invention, the

reagents enabling the antigen/antibody complexes produced to be detected, which can also carry a label or be capable of being recognized in their turn by a labelled reagent, are present. Depending on whether it
5 is desired to carry out an antigen test or a serological test, the kit comprises either the antibodies or the antigens of the invention.

The invention also covers all the nucleotide sequences coding for a polypeptide of the invention, as
10 well as any recombinant nucleic acid containing at least one nucleotide sequence of the invention, inserted into a nucleic acid which is heterologous with respect to the said nucleotide sequence.

The nucleic acid sequences coding for LSA-3 or
15 its immunogenic fragments and corresponding to one of the following definitions form part of the invention:

- (a) the linked succession of nucleotides as depicted in SEQ ID No. 1 of Figure 1, or
- (b) the linked succession of nucleotides depicted in
20 SEQ ID No. 2 of Figure 2,
- (c) a linked succession displaying at least 70% homology with that of Figure 1 or of Figure 2, or
- (d) a linked succession of nucleotides which are complementary to those presented in (a), (b) or (c).

25 The expression "coding for LSA-3" is understood to refer both to the gene depicted in SEQ ID No. 1 of Figure 1 and the cDNA depicted in SEQ ID No. 2 of Figure 2.

The invention relates more especially to a
30 recombinant nucleic acid in which the nucleotide sequence of the invention is preceded by a promoter (in particular an inducible promoter), under the control of which the transcription of the said sequence is capable of being performed, and, where appropriate, followed by
35 a sequence coding for transcription termination signals.

The invention also covers the coding sequence originating from the clone T9/96 depicted in Figure 3 by SEQ ID No. 3.

5 In this sequence, the fragment CT1 lies between nucleotides 67 and 126, the fragment 679 begins at nucleotide 206 and the fragment 729RE lies between nucleotides 547 and 630.

10 Lastly, the invention covers any recombinant vector used especially for the cloning of a nucleotide sequence of the invention, and/or for the expression of the polypeptide encoded by this sequence, and characterized in that it contains a recombinant nucleic acid as defined above in one of its sites which is not essential for its replication.

15 As an example of an abovementioned vector, plasmids, cosmids, phages or viruses may be mentioned.

As such, the invention relates more especially to the plasmid pK 1.2. deposited at the CNCM under the No. I-1573.

20 The subject of the invention is also a method for preparing a polypeptide of the invention, by transformation of a cell host using a recombinant vector of the abovementioned type, followed by the culturing of the cell host thus transformed and the
25 recovery of the polypeptide in the culture medium.

Thus, the invention relates to any cell host transformed by a recombinant vector as defined above, and comprising the regulatory elements permitting the expression of the nucleotide sequence coding for a
30 polypeptide according to the invention.

The invention likewise covers DNA (or RNA) primers which can be used in the context of the synthesis of nucleotide and/or polypeptide sequences of the invention, by the PCR (polymerase chain reaction)
35 technique or any other method known at the present time for amplifying nucleic acids, such as LCR, CPR, ERA, SPA, NASBA, and the like.

The invention relates to any DNA or RNA primer, characterized in that it consists of approximately 10 to 25 nucleotides which are identical or complementary to the first 10 to 25 nucleotides of the nucleotide
5 sequence coding for a peptide sequence according to the invention, or identical to the last 10 to 25 nucleotides of the said sequence.

Thus, the present invention also covers a method for preparing a polypeptide of the invention
10 comprising the following steps:

- where appropriate, the prior amplification by standard techniques of the amount of nucleotide sequences coding for the said polypeptide using two suitably chosen DNA primers,
- 15 - the culturing, in a suitable culture medium, of a cell host previously transformed by a vector containing a nucleic acid according to the invention comprising the nucleotide sequence coding for the said polypeptide, and
- 20 - the recovery from the abovementioned culture medium of the polypeptide produced by the said transformed cell host.

By way of example of DNA or RNA primers according to the invention, the following pairs of
25 sequences may be mentioned:

S1: GTGATGAACTTTTTAATGAATTATTAAA (SEQ ID No. 4)

S2: TGTTGTTCTTGTTGAACACTTTTTACTAA (SEQ ID No. 5)

whose respective positions on the LSA-3/K1 gene depicted in Figure 1 are from 695 to 722 and from 829
30 to 799 (reading in the reverse direction), or the pair:

6.1: GGTATCGAACTGAGGAAATAAAGG (SEQ ID No. 6)

6.2: CATAGCAGGAACATCAACATCCAC (SEQ ID No. 7)

whose respective positions are 2668 to 2692 for 6.1 and 3456 to 3433 for 6.2 (reading in the reverse
35 direction).

The peptides of the invention may also be prepared by the standard techniques of peptide synthesis. This synthesis may be carried out in

homogeneous solution or in the solid phase. For example, use may be made of the technique of synthesis in homogeneous solution described by Houben-Weyl in the work entitled "Methoden der Organischen Chemie" (Methods in Organic Chemistry) edited by E. Wunsch, 5 vol. 15-I and II. Thieme, Stuttgart 1974, or that described by R.D. Merrifield in the paper entitled "Solid phase peptide synthesis" (J. Am. Chem. Soc., 45, 2149-2154).

10 The invention also covers the water-soluble oligomers of the abovementioned monomeric peptides.

Oligomerization can cause an enhancement of the immunogenicity of the monomeric peptides according to the invention. While such numerical information cannot 15 be regarded as limiting, it may nevertheless be mentioned that these oligomers can, for example, contain from 2 to 10 monomer units.

To carry out the oligomerization, use may be made of any polymerization technique commonly used in 20 the peptide field, this polymerization being conducted until an oligomer or polymer containing the requisite number of monomer motifs for acquiring the desired immunogenicity is obtained.

One method of oligomerization or polymerization 25 of the monomer consists in reacting the latter with a crosslinking agent such as glutaraldehyde.

Use may also be made of other oligomerization or coupling methods, for example the one employing successive couplings of monomer units via their 30 carboxy- and amino-terminal functions in the presence of homo- or heterobifunctional coupling agents.

The invention also relates to the conjugates obtained by covalent coupling of the peptides according to the invention (or of the abovementioned oligomers) 35 to physiologically acceptable and non-toxic (natural or synthetic) carrier molecules that enable, in particular, the immunogenicity to be increased, via complementary reactive groups carried, respectively, by

the carrier molecule and the peptide. By way of example of macromolecular carrier molecules or supports which participate in the constitution of the conjugates according to the invention, there may be mentioned
5 natural proteins such as tetanus toxoid, ovalbumin, serum albumins, haemocyanins, tuberculin PPD (PPD: purified protein derivative), and the like.

By way of synthetic macromolecular supports, there may be mentioned, for example, polylysines or
10 poly(DL-alanine)-poly(L-lysine)s.

By way of hydrocarbon or lipid supports, there may be mentioned saturated or unsaturated fatty acids, and preferably C_{16} or C_{18} acids of the oleic or palmitoleic type.

15 To synthesize the conjugates according to the invention, use may be made of methods which are known per se, such as the one described by Frantz and Robertson in *Infect. and Immunity*, 33, 193-198 (1981), or the one described in *Applied and Environmental Microbiology* (October 1981), vol. 42, No. 4, 611-614 by
20 P.E. Kauffman, using the peptide and the appropriate carrier molecule.

The nucleic acids of the invention may be prepared either by a chemical method or by other
25 methods.

A suitable method of preparing the nucleic acids of the invention containing not more than 200 nucleotides (or 200 bp in the case of double-stranded nucleic acids) comprises the following steps:

- 30 - DNA synthesis using the automated β -cyanoethyl-phosphoramidite method described in *Bioorganic Chemistry* 4; 274-325 (1986),
- cloning of the nucleic acids thereby obtained into a suitable vector and recovery of the nucleic acid by
35 hybridization with a suitable probe.

A chemical method of preparation of nucleic acids of length greater than 200 nucleotides has already been described in WO 92/13884.

The invention also relates to diagnostic kits which contain one or more amplification primers specific for the LSA-3 gene and which enable the presence of the gene or of the mRNA to be detected in
5 an individual likely to be infected by P.falciparum.

The invention also covers pharmaceutical or vaccine compositions in which at least one of the products according to the invention is present in combination with solid or liquid, pharmaceutically
10 acceptable excipients suitable for the construction of oral, ocular or nasal dosage forms, or excipients suitable for the construction of dosage forms for rectal administration, or alternatively with gelatinous excipients for vaginal administration. It also relates
15 to isotonic liquid compositions containing at least one of the conjugates according to the invention, suitable for administration to the mucosae, in particular the ocular or nasal or pulmonary mucosae.

Advantageously, the vaccine compositions
20 according to the invention contain, in addition, a vehicle such as polyvinylpyrrolidone which facilitates the administration of the vaccine. In place of polyvinylpyrrolidone, it is possible to use any other type of adjuvant, in the traditional sense which was
25 formerly given to this expression, that is to say a substance which enables a medicinal product to be absorbed more readily or which facilitates its action in the body. By way of examples of other adjuvants of this latter type, there may also be mentioned
30 carboxymethylcellulose, aluminium hydroxides and phosphates, saponin or all other adjuvants of this type which are well known to a person skilled in the art. Lastly, they contain, if necessary, an immunological adjuvant, in particular of the muramyl peptide type.

35 The invention also relates to pharmaceutical compositions containing as active substance at least one of the polyclonal or monoclonal antibodies defined

above, in combination with a pharmaceutically acceptable vehicle.

Additional features of the invention will also become apparent in the examples illustrated with the figures which follow, and show the special features of the molecules of the invention relative to other antigens of the pre-erythrocytic stage of the parasite.

Figure 1 depicts the genomic DNA sequence ID No. 1 of 6152 base pairs of the LSA-3 gene; it originates from the clone K1.2, which itself originates from a Thai isolate.

Figure 2 depicts the cDNA sequence ID No. 2 and the polypeptide sequence of the LSA-3 antigen. The DNA sequence represents 5361 base pairs.

Figure 3 depicts the sequence ID No. 3 of the portion sequenced in the parasite clone T9/96 (1890 base pairs), the upper line being the nucleotide sequence and the lower line the peptide sequence. In this clone, the CT1 sequence lies between nucleotides 67 and 126, the actual fragment DG679 beginning at nucleotide 207. The fragment 729RE lies between nucleotides 547 and 629.

Figure 4a depicts diagrammatically the relative positions of the repeat and non-repeat sequences, the introns and the exons in strains K1 and T9/96, the clones 679 and 729 originating from the latter.

Figure 4b depicts the HCP (hydrophobic cluster plot) of the peptide sequence of the clone DG729.

Figure 5 depicts the amounts of immunoglobulins produced in the serum of chimpanzee Nuria before and after immunization with different LSA-3 peptides.

Figure 6 shows the specific antibody titre of different species of mice immunized either with a peptide or with a corresponding lipopeptide.

Figure 7 shows the inhibition of the sporozoite invasion of liver cells by hyperimmune sera obtained after immunization with different peptides and immunopurified against whole LSA-3.

Figure 8 depicts the comparison of an antigen originating from LSA-3 with two other antigens with respect to type T immunity.

Figure 9 depicts the induction of interferon- γ in the chimpanzees Gerda and Dirk with the peptides originating from the LSA-3 molecule.

Example 1: Cloning and sequencing of the LSA-3 gene

10 1) Sequencing

Initial screening of the gene library originating from the parasite clone T9/96 with the serum of a missionary treated continuously by prophylaxis enabled us to isolate 120 clones corresponding to molecules expressed at the sporozoite and/or liver stage of the P.falciparum cycle. The clone 729S was used as probe to screen a genomic library of the Thai strain K1 already mentioned above, which contains large EcoR I fragments cloned into phage lambda gt10. A 6.85-kilobase insert containing the whole gene was purified from this gene library and recloned into a pUC18 plasmid for sequencing and characterization. In P.falciparum, the genome of which is very rich in bases A:T(80%), this approach is often rendered difficult by the rarity of restriction sites which can be used, and by the instability or even the impossibility of cloning certain fragments when they are inserted into plasmid vectors.

30 The structure of the gene is depicted in Figure 4 and displays the following features:

- a) a mini-exon 1 coding at its 3' end for a hydrophobic signal peptide;
- b) a short intron (168 base pairs) included between consensus splicing donor and acceptor sites;
- 35 c) a second exon of five kilobases which codes for an organized region of 1.8 kilobases, and composed of an arrangement of 7 blocks of 4 amino acids and a 3' hydrophobic region which might correspond to an

anchorage of the glycosylphosphatidylinositol (GPI) type.

5 A detailed investigation of the polymorphism of LSA-3 was carried out by sequencing the clone 679, which contains the bulk of the repeat sequences of the LSA-3 gene and a 1-kilobase portion of the 3' non-repeat fraction, the sequence of this fragment being depicted in Figure 3 between nucleotides 207 and 1890.

10 Comparison of the sequences of the clone 679 originating from P.falciparum clone T9/96, and of the corresponding sequence of LSA-3 originating from the isolate K1, shows that the gene is well conserved, the most significant differences being observed in the repeat region where the blocks of 4 amino acids are
15 well conserved but vary in their number and organization.

In contrast, the non-repeat 5' and 3' portions appear to be especially well conserved, showing up to 100% homology in the 5' region where B and T epitopes
20 have already been identified.

DNA amplifications, in particular by PCR of different P.falciparum strains with 8 primer pairs distributed over the whole of the LSA-3 gene, showed that, except with the ones surrounding the repeat
25 regions, the whole of the genome gives PCR products of similar size, suggesting that the LSA-3 antigen is well conserved.

Various LSA-3 probes, chosen in the repeat and non-repeat regions, were hybridized at low stringency
30 with the DNAs of different species of Plasmodium, and did not enable any gene homologous to LSA-3 to be identified except in the chimpanzee parasite P.reichenowi, confirming the close kinship of this species with P.falciparum.

35 Surprisingly, the antigen analogous to LSA-3 found in P.yoelii, which gives clear immunological cross-reactions at the surface of the sporozoite with antibodies against the fragment 729S, does not appear

to be conserved at the level of the nucleotide sequence. Lastly, comparison of the LSA-3 sequences with the data bases did not reveal any homology with known molecules, except for the repeat region, some of
5 the motifs of which display a strong analogy with the repeats of a *Staphylococcus xylois* gene, but also with two *P.falciparum* antigens, RESA and Pf11.1, which are both expressed during the blood stage of the parasite. This homology is essentially due to the large amount of
10 "Glu-Glu" sequences in these antigens and in the repeats of LSA-3.

2) Cloning

The insert DG729 and other regions of exon 2 of the strain K1 were cloned into a prokaryotic expression
15 vector pGEX, a vector marketed by the company InVitrogen Corp (San Diego USA). This vector produces a fusion protein with the *Schistosoma mansoni* glutathione S-transferase (GST), and enables the recombinant proteins to be purified readily by affinity for
20 glutathione-agarose beads. The expression peptides from these vectors are designated:

- for the whole LSA-3 protein: REC protein,
- or for the fragment 729S:729PGEX.

Attempts at cloning other fragments, in
25 particular the fragment 1-5 3NSREP, 3NFREP, 5NR and 5SNREP, caused difficulties related either to the cloning or to the production and purification of the proteins in sufficient amounts for immunization experiments.

30 Only the fragments 729, NN and 3PC enabled corresponding recombinant polypeptides to be produced and purified in sufficient amounts for analysis of the antigenicity of the molecule.

Example 2: Comparison of the antibody responses of chimpanzee Nuria before and after immunization with different peptides

5 Figure 5 depicts the amounts of immunoglobulins present in the serum of chimpanzee Nuria before and after immunization with the peptides 729NR1 and 729RE, and the lipopeptides 729NR2 and CT1.

This experiment shows the superiority as regards B immunity of the R antigen, most particularly
10 when it is conjugated to a lipid residue.

Figure 6 shows that the level of specific antibodies measured by ELISA against the peptide 729NR2 in mice immunized with either the peptide 729NR1 or the lipopeptide 729NR2 is markedly higher when the lipopeptide is used, irrespective of the species of mouse.
15

Example 3: Effects of the antibodies against the LSA-3 peptides on the inhibition of the entry of sporozoites in mice

The techniques used to prepare the primary
20 hepatocyte cultures, the sporozoites, the antibodies and the indirect fluorescence test are described in detail by S. Mellouk et al., Bulletin of the World Health Organization, 68: 52-59, 1990. The table below compares the results obtained in immunofluorescence,
25 either with antibodies against the fragment 679 or with antibodies obtained against fragments originating from other peptides. The left-hand column shows the number of schizonts detected after 48 h of culture in hepatocytes of Balb/c mice infected by P.yoelii and the
30 right-hand column the same parameters after infection by P.berghei.

Antibody clones	P.yoelii			P.berghei		
	IFA	No. of LS at 48 h		IFA	No. of LS at 48 h	
Control		a)	b)			
679	++	88	110	-	119	108
	++		0	-		47
	++		0	-		ND
679	++	1		-	105	
679b	++	1		-	133	
679c	++	1		-	30	
32	++	8		±	103	
222	+		5	±		26
667	++	276	143	ND	502	
362	+	3				
493	++	55		ND	508	
α P.b. CSP Mab			82	+++		30
α P.y. CSP Mab	+++		171		138	

It is clearly apparent that the antibody against the peptide 679 has an almost complete inhibitory effect on the number of what they [sic] observed at 48 h in the liver cells. Likewise, Figure 7 shows the inhibition of the sporozoite invasion of liver cells by hyper human [sic] sera obtained after immunization with different peptides and immunopurified against whole LSA-3.

Example 4: Cytotoxicity test against the peptide 729NRII in the chimpanzee Gerda

The chimpanzee Gerda was immunized via the i.v. route with the lipopeptide 729NRII originating from the LSA-3 antigen. Blood is drawn 9 days after the 4th injection. The PBMCs were incubated in vitro with 5 µg/ml of the peptide 729NRII (addition of recombinant IL-2, 10 U/ml, on day 3). On day 15, the cytotoxic activity was studied against autologous blasts generated with PHA at a concentration of 0.5 µg/ml. The blasts were preincubated overnight with 5 µg/ml of the peptide 729NRII, and with a control peptide, namely RESA, or without a peptide. The peptides are not added

during the test (8 hours). The number of targets per well is 5000.

PBMCs from Gerda incubated for the same period with 5 µg/ml of a control peptide or the peptide 729NRI (originating from the same antigen) do not bring about the lysis of autologous blasts preincubated or otherwise with the above peptides.

Figure 8 shows the results obtained for an E/T (effector to target) ratio varying from 12 to 0.03. It is seen that the target cells presensitized with the peptide 729NRII are lysed in the presence of effector cells, indicating a cytotoxic T type immune response specific to this antigen.

The lipopeptide NRII injected via the i.v. route is capable, without adjuvant, of inducing a specific cytotoxic response.

Example 5: Effect of the peptide NRI on interferon-γ production

Interferons have been shown to have an inhibitory activity in the development of P.falciparum in human hepatocytes in culture (Sylvie Mellouk et al., The Journal of Immunology, vol. 139 No. 12: 41-92, 41-95, 1987). The results obtained with the peptides of the invention are as follows:

The chimpanzee Gerda, immunized with the polypeptide NR2 and boosted with the recombinant DG729, carries PBMCs capable of secreting high levels of IFN-γ in the presence of the LSA-3 peptides, especially the peptide 729NRI. The result was confirmed in the chimpanzee Dirk, immunized with the same protein. The chimpanzee BRAM, an unimmunized control, does not show any interferon in the blood against the LSA-3 peptides.

The sequences ID n° 1 to 3 are depicted in figures 1 to 3.

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTGATGAACT TTTAATGAA TTATTAAA

28

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGTTGTTCTT GTTGAACACT TTTTACTAA

29

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGTATCGAAA CTGAGGAAAT AAAGG

25

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CATAGCAGGA ACATCAACAT CCAC

24

CLAIMS

1. Polypeptide molecules containing at least 10 consecutive amino acids of the amino acid sequence shown in Figure 2, the following polypeptides being excluded:
 - RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQOEQQHNV
 - VEESVEENDEESVEENVEENVENNDDGSVASSVEESIASSVDESIDSSIE-ENVAPTVEEIVAPTVEEIVAPSVVEKCAPSVVEESVAPSVVEESVAEMLKER (729S)
 - RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQOEQQHN
 - DELFNELLNSVDVNGEVKENILEESQ, (NRI)
 - LEESQVNDDIFNSLVKSVQOEQQHNV, (NRII)
 - VESVAPSVVEESVAPSVVEESVAENVESV. (729RE)
2. Molecules according to Claim 1, characterized in that they contain at least 20 consecutive amino acids of the said sequence.
3. Molecules according to Claim 2, characterized in that they contain at least 50 consecutive amino acids of the said sequence.
4. Polypeptide molecule displaying at least 70% homology with one of the molecules of any one of Claims 1 to 3.
5. Polypeptide molecule, characterized in that it displays at least 70% homology with the following sequence:

Leu Leu Ser Asn Ile Glu Glu Pro Lys Glu Asn Ile Ile Asp Asn Leu Leu Asn Asn Ile (CT1).
6. Polypeptide molecule according to one of Claims 1 to 4, characterized in that it displays at least 70% homology with the sequence depicted in Figure 3.
7. Immunogenic composition, characterized in that it contains at least one polypeptide molecule according to any one of Claims 1 to 6 and at least one pharmaceutical vehicle.

8. Antimalarial vaccine composition containing, among other immunogenic principles, a polypeptide molecule according to one of Claims 1 to 6.

5 9. Vaccine composition according to Claim 8, characterized in that it contains, in addition, a molecule containing at least one epitope and which originates from the group consisting of the LSA-1, SALSA or STARP molecules.

10 10. Composition according to Claim 9, characterized in that it contains at least two immunogens, the first being chosen from the following polypeptides:

- that of Figure 2,
- NRI,
- NRII,

15 and the second being chosen from the group consisting of SALSA, SALSA I and SALSA II.

11. Polyclonal or monoclonal antibodies which specifically recognize the polypeptide molecules according to any one of Claims 1 to 6.

20 12. Method of in vitro diagnosis of malaria in an individual likely to be infected by P. falciparum, which comprises the bringing of a tissue or biological fluid taken from an individual into contact with a molecule according to one of Claims 1 to 8, under
25 conditions permitting an immunological reaction, said polypeptide molecule and antibodies possibly present in the tissue or the biological fluid, and the in vitro detection of the antibody gene [sic] complexes possibly formed.

30 13. Method according to Claim 12, characterized in that the tissue or biological fluid is brought into contact with a mixture of polypeptide molecules responding [sic] to one of Claims 1 to 6 and other molecules originating from antigens of the sporozoite
35 stage, namely LSA-1, SALSA or STARP.

14. Method of in vitro diagnosis of malaria in an individual likely to be infected by P. falciparum,

characterized in that it comprises the bringing of a tissue or biological fluid taken from an individual into contact with antibodies according to Claim 11, under conditions permitting an immunological reaction
5 in vitro between the said antibodies and the proteins specific to P. falciparum which are possibly present in the biological tissue, and the in vitro detection of the antigen/antibody complexes possibly formed.

15. Kit for the in vitro diagnosis of malaria according to Claim 12 or 13, characterized in that it comprises at least one or several molecules according to one of Claims 1 to 6,
10 the reagents for making up the appropriate medium for the reaction,
15 the reagents enabling the antigen/antibody complexes produced by the immunological reaction to be detected, it also being possible for these reagents to carry a label or to be capable of being recognized in their turn by a labelled reagent, more especially in the case
20 where the abovementioned polypeptide molecule is not labelled.

16. Kit for the in vitro diagnosis of malaria, characterized in that it comprises:
- antibodies according to Claim 11,
25 - the reagents for making up the appropriate medium for carrying out the immunological reaction,
- the reagents enabling the antigen/antibody complexes produced by the immunological reaction to be detected, it also being possible for these reagents to carry a
30 label or to be capable of being recognized in their turn by a labelled reagent, more especially in the case where the abovementioned antibodies are not labelled.

17. Use of a polypeptide molecule according to one of Claims 1 to 6 in the preparation of an antimalarial
35 vaccine.

18. Use of one or more polyclonal or monoclonal antibodies according to Claim 11 for the preparation of

a medicinal product intended for the treatment of malaria.

19. Pharmaceutical composition containing as active substance one or more polyclonal or monoclonal
5 antibodies according to Claim 11, in combination with an acceptable pharmaceutical vehicle.

20. Nucleic acid sequence, characterized by one of the following sequences:

(a) the linked succession of nucleotides as depicted in
10 SEQ ID No. 1 of Figure 1, or

(b) the linked succession of nucleotides depicted in SEQ ID No. 2 of Figure 2,

(c) a linked succession displaying at least 70% homology with that of Figure 1 or of Figure 2, or

15 (d) a linked succession of nucleotides which are complementary to those presented in (a), (b) or (c).

21. Nucleic acid according to Claim 20, containing a sequence coding for a polypeptide molecule according to one of Claims 1 to 6.

20 22. Recombinant vector for the cloning of a nucleotide sequence according to Claim 20 or Claim 21 and/or the expression of a polypeptide encoded by the abovementioned sequence, containing the said sequence in one of the sites which is not essential for its
25 replication, the said vector being, in particular, of the plasmid, cosmid or phage type.

23. Vector according to Claim 22, characterized in that it is a plasmid deposited at the CNCM under the No. I-1573 and referenced pK1.2.

ABSTRACT

Polypeptide molecules containing at least 10 consecutive amino acids of the amino acid sequence shown in figure 2, representing the LSA3 antigen, the following peptides being excluded:

- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHNV
- VEESVEENDEESVEENVEENVENNDDGSVASSVEESIASSVDESIDSSIE-
ENVAPTVEEIVAPTVEEIVAPSVVEKCAPSVVEESVAPSVVEESVAEMLKER
10 (729S)
- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHN
- DELFNELLNSVDVNGEVKENILEESQ, (NRI)
- LEESQVNDDIFNSLVKSVQQEQQHNV, (NRII)
- VESVAPSVVEESVAPSVVEESVAENVESSV. (729RE)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6152 base pairs
 (B) TYPE: nucleotide
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATTIATTTAT TTTIATTGTT TIATTTCTTT TTTTCTTIA AATGTATAT TTATAAATAT	60
TTTAAAAAGT TAGAAATGA CAAATAGTAA TTACAAATCA AATAATAAAA CATATAATGA	120
AAATAATAAT GAAGAAATAA CTACCATATT TAATAGAACA AATATGAATC GGATAAAAAA	180
ATGTCATATG AGAGAAAAAA TAAATAAGTA CTTTTTTTTC ATCAAAATTT TGACATGCAC	240
CATTTTAAATA TGGCCTGTAC AATATGATAA TAACGTAAGA TAAAAAACTA AATAATAAAT	300
ATAAATAAAA AAAAAAAAAA AAAAAAAAAA ATCAAGTATA TAGTATGTAT AATATATATA	360
TATATATATA TATATATATA TATATATATA TATTTATTTT TATTTATTTA TTAATTTTTT	420
TTTTTTTATA TIATCTTTTT AGTCTGATAT AAACAAGAGT TGGAAAAAAA ATACGTATGT	480
AGATAAGAAA TTGAATAAAC TATTTAACAG AAGTTTACGA GAATCTCAAG TAAATGGTCA	540
ATTAGCTAGT GAAGAAGTAA AGCAAAAAAT TCTTCACTTA TTACAAGAAG GAAATACATT	600
AACTGAAAGT GTAGATGATA ATAAAAATTT AGAAGAAGCC GAAGATATAA AGCAAAATAT	660
CTIATIAAGT AATATAGAAG AACCAAAAGA AATATTIATT GACAATTTAT TAAATAATAT	720
TGGACAAAT TCAGAAAAAC AAGAAAGTGT ATCAGAAAAAT GTACAAGTCA GTGATCAACT	780
TTTAAATGAA TIATTAAATA GTCTAGATGT TAATGGAGAA GTAAAAGAAA ATATTTTCCA	840
CGAAAGTCAA GTTAATGAGC ATATTTTTTAA TAGTTTAGTA AAAAGTCTTC AACAGAACA	900
ACAAGACAAT GTTGAAGAAA AAGTTCAGCA AAGTGTAGAA GAAATCACC AACAAAGTGT	960
AGAAGAAAAT GTACAAGAAA ATGTACAAGA AATCACCAC CGAAGTGTAG CCTCAAGTGT	1020
TGAAGAAAGT ATAGCTTCAA GTCTTCATGA AAGTATAGAT TCAAGTATTG AACAAAATGT	1080
AGCTCCAAGT GTTGAAGAAA TCGTAGCTCC AAGTCTTCTA GAAAGTCTGG CTCCAAGTGT	1140

FIGURE 1/A

TGAAGAAAGT	GTAGAAGAAA	ATGTTGAAGA	AAGTGTAGCT	GAAAATGTTG	AAGAAAGTGT	1200
AGCTGAAAAT	GTTGAAGAAA	CTGTAGCTGA	AAATGTTGAA	GAAAGTGTAG	CTGAAAATGT	1260
TGAAGAAATC	GTAGCTCCAA	CTGTTGAAGA	AATCGTAGCT	CCAACTGTTG	AAGAAATGT	1320
AGCTCCAAGT	GTTGTAGAAA	CTGTGGCTCC	AAGTGTGAA	GAAAGTGTAG	AAGAAAATGT	1380
TGAAGAAAGT	GTAGCTGAAA	ATGTTGAAGA	AAGTGTAGCT	GAAAATGTTG	AAGAAAGTGT	1440
AGCTGAAAAT	GTTGAAGAAA	CTGTAGCTGA	AAATGTTGAA	GAAAGTGTAG	CTGAAAATGT	1500
TGAAGAAATC	GTAGCTCCAA	CTGTTGAAGA	AATCGTAGCT	CCAACTGTTG	AAGAAATGT	1560
AGCTCCAAGT	GTTGTAGAAA	CTGTGGCTCC	AAGTGTGAA	GAAAGTGTAG	AAGAAAATGT	1620
TGAAGAAAGT	GTAGCTGAAA	ATGTTGAAGA	AAGTGTAGCT	GAAAATGTTG	AAGAAAGTGT	1680
AGCTGAAAAT	GTTGAAGAAA	CTGTAGCTGA	AAATGTTGAA	GAAAGTGTAG	CTGAAAATGT	1740
TGAAGAAAGT	GTAGCTGAAA	ATGTTGAAGA	AAGTGTAGCT	GAAAATGTTG	AAGAAATGCT	1800
AGCTCCAAGT	GTTGAAGAAA	TCGTAGCTCC	AAGTGTGAA	GAAATGTAG	CTCCAAGTGT	1860
TGTAGAAAGT	GTGCTCCAA	CTGTTGAAGA	AAGTGTAGAA	GAAAATGTTG	AAGAAAGTGT	1920
AGCTGAAAAT	GTTGAAGAAA	CTGTAGCTGA	AAATGTTGAA	GAAAGTGTAG	CTGAAAATGT	1980
TGAAGAAAGT	GTAGCTGAAA	ATGTTGAAGA	AATCGTAGCT	CCAACTGTTG	AAGAAATGCT	2040
AGCTCCAAGT	GTTGAAGAAA	TTGTAGCTCC	AAGTGTGTA	GAAAGTGTG	CTCCAAGTGT	2100
TGAAGAAAGT	GTAGAAGAAA	ATGTTGAAGA	AAGTGTAGCT	GAAAATGTTG	AAGAAAGTGT	2160
AGCTGAAAAT	GTTGAAGAAA	CTGTAGCTGA	AAATGTTGAA	GAAATCGTAG	CTCCAAGTGT	2220
TGAAGAAATC	GTAGCTCCAA	CTGTTGAAGA	AATTGTAGCT	CCAAGTGTG	TAGAAAGTGT	2280
CGCTCCAAGT	GTTGAAGAAA	GTGTAGAAGA	AAATGTTGAA	GAAAGTGTAG	CTGAAAATGT	2340
TGAAGAAAGT	GTAGCTGAAA	ATGTTGAAGA	AAGTGTAGCT	GAAAATGTTG	AAGAAAGTGT	2400
AGCTGAAAAT	GTTGAAGAAA	TCGTAGCTCC	AAGTGTGAA	GAAATCGTAG	CTCCAAGTGT	2460
TGAAGAAATT	GTAGCTCCAA	CTGTTGTAGA	AAGTGTGGCT	CCAAGTGTG	AAGAAAGTGT	2520
AGAAGAAAAT	GTTGAAGAAA	CTGTAGCTGA	AAATGTTGAA	GAAAGTGTAG	CTGAAAATGT	2580
TGAAGAAAGT	GTAGCTGAAA	ATGTTGAAGA	AAGTGTAGCT	CCAACTGTTG	AAGAAATGT	2640
AGCTCCAAGT	GTTGAAGAAA	CTGTAGCTCC	AAGTGTGAA	GAAAGTGTG	CTGAAAACGT	2700
TGCAACAAAT	TTATCAGACA	ATCTTTTAAG	TAATTTATTA	GCTGCTATCG	AAACTCAGGA	2760

FIGURE 1/B

AATAAAGGAC ACTATATTAA ATGAGATAGA AGAAGTAAAA GAAAATGTAG TCACCACAAT	2820
ACTAGAAAAC GTAGAAGAAA CTACAGCTGA AAGTGTAAGT ACTTTTAGTA ACATATTAGA	2880
GGAGATACAA GAAAATACTA TTACTAATGA TACTATAGAG GAAAATTAG AAGAAGTCCA	2940
CGAAAATGTA TTAAGTCCCG CTTTAGAAAA TACCCAAAGT GAAGAGGAAA AGAAAGAAGT	3000
AATAGATGTA ATTGAAGAAG TAAAGAAGA GGTGGCTACC ACTTTAATAG AAAGTGTGGA	3060
AGAGGCAGAA GAAAAGACCG CAAATACAAT TACCGAAATA TTTGAAAATT TAGAAGAAAA	3120
TGCAGTAGAA ACTAATGAAA ATGTTGCAGA CAATTTAGAG AAATTAAAGG AAAGTGTATT	3180
TAATACTGTA TTAGATAAAG TAGAGGAAAC AGTAGAAATT AGCGGAGAAA GTTTAGAAAA	3240
CAATGAAATG GATAAAGCAT TTTTACTGA AATATTGAT AATGTAAAAG CAATACAAGA	3300
AAATTIATTA ACAGGTATGT TTCGAAGTAT AGAAACCAAT ATAGTAATCC AATCAGAAGA	3360
AAAGGTTGAT TTCAATGAAA ATGTGCTTAG TTCCATTITA GATAATATAG AAAATATGAA	3420
AGAAGGTTTA TTAAATAAAT TAGAAAATAT TTCAACTACT GAAGCTGTTT AAGAAAGTGT	3480
AACTCAACAT GTAGAACAAA ATGTATATGT CGATGTTGAT GTTCTGCTTA TGAAGATCA	3540
ATTTTAGGA ATATTAAATG AGGCAGGAGG GTTGAAGAA ATGTTTTTA ATTTGGAAGA	3600
TGTATTTAAA AGTGAAGTGT ATGTAATTAC TGTAGAAGAA ATTAAGGATG AAGCGGTTCA	3660
AAAAGAGCTA GAAAAGAAA CTGTTAGTAT TATTGAAGAA ATGGAAGAAA ATATTGTAGA	3720
TGTATTAGAG GAAGAAAAAG AAGATTAAAC AGACAAGATG ATAGATCCAG TAGAAGAAAC	3780
CATAGAAATA TCTTCAGATT CTAAGAAGA AACTGAATCT ATTAAAGATA AAGAAAAAGA	3840
TGTTTCAGTA GTTGTGGAAG AAGTTCAAGA CAATGATATG GATGAAAGTG TTCAGAAAGT	3900
TTTGAATTC AAAAATATCG AAGAGGAGTT AATGAAGCAT GCTGTTGAAA TAAATGACAT	3960
TACTAGCAAA CTTATTGAAG AAAGTCAAGA GTTAAATGAA GTAGAAGCAG ATTTAATAAA	4020
AGATATCGAA AAATTAAAG AATTAGAAA AGCATTATCA GAAGATTCTA AAGAAATAAT	4080
AGATGCAAAA GATGATACAT TAGAAAAAGT TATTCAACAG GAACATGATA TAAGCAGGAC	4140
GTGGATGAA GTTGTAGAAT TAAAGATGT CGAAGAAGAC AAGATCGAAA AAGTATCTGA	4200
TTTAAAGAT GTTGAAGAAG ATATATTAAA AGAAGTAAAA GAAATCAAAG AACTTGAAGG	4260
TGAAATTTTA GAAGATTATA AAGAATTAAA AACTATTGAA ACAGATATTT TAGAAGAGAA	4320
AAAAGAAATA GAAAAGATC ATTTTGAAGA ATTCCAAGAA GAAGCTGAAG AAATAAAGA	4380

FIGURE 1/C.

TCTTGAAGCA GATATATTAA AAGAAGTATC TTCATTAGAA GTTGAAGAAG AAAAAAATT	4440
AGAAGAAGTA CACGAATTAA AAGAAGAGGT AGAAGATATA ATAAGTGGTG ATGCCCATAT	4500
AAAAGGTTTG GAAGAAGATG ATTTAGAAGA AGTAGATGAT TTAAAAGGAA GTATATTAGA	4560
CATGTTAAAG GCAGATATGC AATTACCGCA TATCGATAAG GAAAGTTIAG AAGATGTAAC	4620
AACAAAACCTT GCAGAAAGAG TTGAATCCTT AAAAGATGTT TTATCTAGTG CATTAGCCAT	4680
GGATGAAGAA CAAATGAAAA CAAGAAAAAA ACCTCAAAGA CCTAAGTTGC AACAAGTATT	4740
ATTAAAAGAA GAGCTTAAAG AAGAACCAAA CAAAAAATA ACAAAAAAGA AAGTAAAGTT	4800
TGATATTAAG GATAAGGAAC CAAAAGATGA AATAGTAGAA GTTGAAATGA AAGATGAAGA	4860
TATACAAGAA GATGTAGAAG AAGATATAGA AGAAGATATA GAAGAAGATA AAGTTGAAGA	4920
TATAGATGAA GATATAGATG AAGATATAGG TGAAGACAAA GATGAAGTTA TAGATTAAAT	4980
AGTCCAAAAA GAGAAACCGA TTGAAAAGCT TAAAGCGAAA AAGAAAAAAT TAGAAAAAAA	5040
AGTTCAAGAA GGTGTIAGTG GTCTTAAAAA ACACCTAGAC CAAGTAATGA AATATGTTCA	5100
AAAAATTGAT AAAGAAGTTG ATAAAGAAGT ATCTAAAGCT TTAGAATCAA AAAATGATGT	5160
TACTAATGTT TTAAACAAA ATCAAGATTI TTTTAGTAAA GTTAAAAACT TCGTAAAAAA	5220
ATATAAGTA TTTGCTCCAC CATTCAATATC TCCCGTTGCA GCATTTCGAT CATATGTAGT	5280
TGGCTTCCTT ACATTTTCTT TATTTTCATC ATGTGTAAAC ATAGCTTCTT CAAGTTAGTT	5340
ATATCAAAA GTTGACAAAA CTATAAATAA AATAAGGAG AGACCGTTTT ATTCATTGCT	5400
ATTGATATC TTAAAGAATT TAAACATTA TTACAACAA ATGAAAGAAA AATTTAGTAA	5460
AGAAAAAAT AATAATGTAA TAGAAGTAAAC AAACAAGCT CAGAAAAAAG GTAATGTACA	5520
GCTAACAAAT AAAACCGAGA AAACAATAA AGTTGATAAA AATAATAAAG TACCGAAAAA	5580
AAGAACAACG CAAAAATCAA AATAAAAAAT TGCAGAACAC TGAATGATT GGAGCGAACA	5640
ATAAAATTAA TCGATAAAAA ATATAAAAT GTATATATTA TGTAAATATA TATAAATAAA	5700
TAAATAAATA CATACATATA TATATATATA TATATCTATC TTTTIACAAA ATTTTAAAT	5760
TTTAAATTT ATATATATTA ATATTIATAT TTTTCCATAT ATAATTTTAT TTTCAATATT	5820
TTATTTTAA TTATAAATGT TTTTACAGA GTTTATGTTT TTAATTAAT ATATAGATTT	5880
CTGTAAGAAA CTGTATATTA TTCATACGAT ATATGTAATA TTAATTATTT GTGTTTIATT	5940
AAAATTTATA TTATATAATA TATATATATA TATATATGTA TATATATTAG AAGATAAAAA	6000

FIGURE 1/D

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TTIAGCTTAT TTIGCTTGT ATGCAAATAA GCTTTTTTTT TTTTTTTTTT TTTTTTTTC	6060
ATAIAAACGA TGTITTAATT TTAATTTTAA ATATTTTATA TAAATATTT TTCCTAAAAA	6120
AAAAAAAAAT TAAAAAAAAAC TTATATTTCG AA	6152

FIGURE 1/B

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5361 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..5361

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATG ACA AAT AGT AAT TAC AAA TCA AAT AAT AAA ACA TAT AAT GAA AAT	48
Met Thr Asn Ser Asn Tyr Lys Ser Asn Asn Lys Thr Tyr Asn Glu Asn	
1 5 10 15	
AAT AAT GAA CAA ATA ACT ACC ATA TTT AAT AGA ACA AAT ATG AAT CCC	96
Asn Asn Glu Gln Ile Thr Thr Ile Phe Asn Arg Thr Asn Met Asn Pro	
20 25 30	
ATA AAA AAA TGT CAT ATG AGA GAA AAA ATA AAT AAG TAC TTT TTT TTC	144
Ile Lys Lys Cys His Met Arg Glu Lys Ile Asn Lys Tyr Phe Phe Leu	
35 40 45	
ATG AAA ATT TTC ACA TGC ACC ATT TTA ATA TGG GCT GTA CAA TAT GAT	192
Ile Lys Ile Leu Thr Cys Thr Ile Leu Ile Trp Ala Val Gln Tyr Asp	
50 55 60	
AAT AAC TCT GAT ATA AAC AAG AGT TGG AAA AAA AAT ACC TAT GTA CAT	240
Asn Asn Ser Asp Ile Asn Lys Ser Trp Lys Lys Asn Thr Tyr Val Asp	
65 70 75 80	
AAG AAA TTG AAT AAA CTA TTT AAC AGA AGT TTA GGA GAA TGT CAA GTA	288
Lys Lys Leu Asn Lys Leu Phe Asn Arg Ser Leu Gly Glu Ser Gln Val	
85 90 95	
AAT GCT GAA TTA GCT AGT GAA GAA CTA AAG GAA AAA ATT CTT CAC TTA	336
Asn Gly Glu Leu Ala Ser Glu Glu Val Lys Glu Lys Ile Leu Asp Leu	
100 105 110	
TTA GAA CAA GGA AAT ACA TTA ACT GAA AGT GTA GAT GAT AAT AAA AAT	384
Leu Glu Glu Gly Asn Thr Leu Thr Glu Ser Val Asp Asp Asn Lys Asn	
115 120 125	

FIGURE 2/A

TIA GAA GAA GCC GAA GAT ATA AAG GAA AAT ATC TIA TIA AGT AAT ATA Leu Glu Glu Ala Glu Asp Ile Lys Glu Asn Ile Leu Leu Ser Asn Ile 130 135 140	432
GAA GAA CCA AAA GAA AAT ATT ATT GAC AAT TIA TIA AAT AAT ATT GCA Glu Glu Pro Lys Glu Asn Ile Ile Asp Asn Leu Leu Asn Asn Ile Gly 145 150 155 160	480
CAA AAT TCA GAA AAA CAA GAA AGT GTA TCA GAA AAT GTA CAA GTC AGT Gln Asn Ser Glu Lys Gln Glu Ser Val Ser Glu Asn Val Gln Val Ser 165 170 175	528
GAT GAA CTT TTT AAT GAA TTA TTA AAT AGT GTA GAT GTT AAT GCA GAA Asp Glu Leu Phe Asn Glu Leu Leu Asn Ser Val Asp Val Asn Gly Glu 180 185 190	576
GTA AAA CAA AAT ATT TTG GAG GAA AGT CAA GTT AAT GAC GAT ATT TTT Val Lys Glu Asn Ile Leu Glu Glu Ser Gln Val Asn Asp Asp Ile Phe 195 200 205	624
AAT ACT TTA GTA AAA AGT GTT CAA CAA GAA CAA CAA CAC AAT GTT GAA Asn Ser Leu Val Lys Ser Val Gln Gln Glu Gln Gln His Asn Val Glu 210 215 220	672
GAA AAA GTT CAA GAA AGT GTA GAA GAA AAT GAC CAA GAA AGT GTA GAA Glu Lys Val Glu Glu Ser Val Glu Glu Asn Asp Glu Glu Ser Val Glu 225 230 235 240	720
GAA AAT GTA CAA GAA AAT GTA GAA GAA AAT GAC GAC GCA AGT GTA GCC Glu Asn Val Glu Glu Asn Val Glu Glu Asn Asp Asp Gly Ser Val Ala 245 250 255	768
TCA AGT GTT GAA GAA AGT ATA GCT TCA AGT GTT GAT CAA AGT ATA GAT Ser Ser Val Glu Glu Ser Ile Ala Ser Ser Val Asp Glu Ser Ile Asp 260 265 270	816
TCA AGT ATT GAA GAA AAT GTA CCT CCA AGT GTT GAA GAA ATC GTA GCT Ser Ser Ile Glu Glu Asn Val Ala Pro Thr Val Glu Glu Ile Val Ala 275 280 285	864
CCA AGT GTT GTA GAA AGT GTC GCT CCA AGT GTT GAA GAA AGT GTA GAA Pro Ser Val Val Glu Ser Val Ala Pro Ser Val Glu Glu Ser Val Glu 290 295 300	912
GAA AAT GTT GAA GAA AGT GTA GCT CAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 305 310 315 320	960
GAA AAT GTT CAA GAA AGT GTA CCT CAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 325 330 335	1008

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CAA AAT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA GAA ATC GTA GCT Glu Asn Val Glu Glu Ile Val Ala Pro Thr Val Glu Glu Ile Val Ala 340 345 350	1056
CCA ACT GTT GAA GAA ATT GTA GCT CCA AGT GTT GTA GAA AGT GTC GCT Pro Thr Val Glu Glu Ile Val Ala Pro Ser Val Val Glu Ser Val Ala 355 360 365	1104
CCA AGT GTT GAA GAA ACT GTA GAA GAA AAT GTT GAA GAA AGT GTA GCT Pro Ser Val Glu Glu Ser Val Glu Glu Asn Val Glu Glu Ser Val Ala 370 375 380	1152
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 385 390 395 400	1200
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 405 410 415	1248
GAA AAT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA GAA ATC GTA GCT Glu Asn Val Glu Glu Ile Val Ala Pro Thr Val Glu Glu Ile Val Ala 420 425 430	1296
CCA ACT GTT GAA GAA ATT GTA GCT CCA AGT GTT GTA GAA AGT GTC GCT Pro Thr Val Glu Glu Ile Val Ala Pro Ser Val Val Glu Ser Val Ala 435 440 445	1344
CCA AGT GTT GAA GAA AGT GTA GAA GAA AAT GTT GAA GAA AGT GTA GCT Pro Ser Val Glu Glu Ser Val Glu Glu Asn Val Glu Glu Ser Val Ala 450 455 460	1392
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 465 470 475 480	1440
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 485 490 495	1488
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 500 505 510	1536
GAA AAT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA GAA ATC GTA GCT Glu Asn Val Glu Glu Ile Val Ala Pro Thr Val Glu Glu Ile Val Ala 515 520 525	1584
CCA ACT GTT GAA GAA ATT GTA GCT CCA AGT GTT GTA GAA AGT GTC GCT Pro Thr Val Glu Glu Ile Val Ala Pro Ser Val Val Glu Ser Val Ala 530 535 540	1632

FIGURE 2/C

CCA AGT GTT GAA GAA AGT GTA GAA CAA AAT GTT GAA GAA AGT GTA GCT Pro Ser Val Glu Glu Ser Val Glu Glu Asn Val Glu Glu Ser Val Ala 545 550 555 560	1680
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 565 570 575	1728
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA ATC GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ile Val Ala 580 585 590	1776
CCA ACT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA GAA ATT GTA GCT Pro Thr Val Glu Glu Ile Val Ala Pro Thr Val Glu Glu Ile Val Ala 595 600 605	1824
CCA AGT GTT GTA GAA AGT GTG GCT CCA AGT GTT GAA GAA AGT GTA GAA Pro Ser Val Val Glu Ser Val Ala Pro Ser Val Glu Glu Ser Val Glu 610 615 620	1872
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 625 630 635 640	1920
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA ATC GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ile Val Ala 645 650 655	1968
CCA ACT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA GAA ATT GTA GCT Pro Thr Val Glu Glu Ile Val Ala Pro Thr Val Glu Glu Ile Val Ala 660 665 670	2016
CCA AGT GTT GTA GAA AGT GTG GCT CCA AGT GTT GAA GAA AGT GTA GAA Pro Ser Val Val Glu Ser Val Ala Pro Ser Val Glu Glu Ser Val Glu 675 680 685	2064
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 690 695 700	2112
GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 705 710 715 720	2160
GAA AAT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA GAA ATC GTA GCT Glu Asn Val Glu Glu Ile Val Ala Pro Thr Val Glu Glu Ile Val Ala 725 730 735	2208
CCA ACT GTT GAA GAA ATT GTA GCT CCA AGT GTT GTA GAA AGT GTG GCT Pro Thr Val Glu Glu Ile Val Ala Pro Ser Val Val Glu Ser Val Ala 740 745 750	2256

FIGURE 2/D.

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CCA AGT GTT GAA GAA AGT GTA GAA GAA AAT GTT GAA GAA AGT GTA GCT Pro Ser Val Glu Glu Ser Val Glu Glu Asn Val Glu Glu Ser Val Ala 755 760 765	2304
GAA AAT GTT GAA GAA AGT GTA GCT CAA AAT GTT GAA GAA AGT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala 770 775 780	2352
GAA AAT GTT GAA GAA AGT GTA GCT CCA ACT GTT GAA GAA ATT GTA GCT Glu Asn Val Glu Glu Ser Val Ala Pro Thr Val Glu Glu Ile Val Ala 785 790 795 800	2400
CCA AGT GTT GAA GAA AGT GTA GCT CCA AGT GTT GAA GAA AGT GTT GCT Pro Ser Val Glu Glu Ser Val Ala Pro Ser Val Glu Glu Ser Val Ala 805 810 815	2448
GAA AAC GTT GCA ACA AAT TTA TCA GAC AAT GTT TTA AGT AAT TTA TTA Glu Asn Val Ala Thr Asn Leu Ser Asp Asn Leu Leu Ser Asn Leu Leu 820 825 830	2496
GCT GCT ATC CAA ACT GAG GAA ATA AAG GAC AGT ATA TTA AAT GAG ATA Gly Gly Ile Glu Thr Glu Glu Ile Lys Asp Ser Ile Leu Asn Glu Ile 835 840 845	2544
GAA GAA GTA AAA GAA AAT GTA GTC ACC ACA ATA CTA GAA AAC GTA GAA Glu Glu Val Lys Glu Asn Val Val Thr Thr Ile Leu Glu Asn Val Glu 850 855 860	2592
GAA ACT ACA GCT GAA AGT GTA ACT ACT TTT AGT AAC ATA TTA GAG GAG Glu Thr Thr Ala Glu Ser Val Thr Thr Phe Ser Asn Ile Leu Glu Glu 865 870 875 880	2640
ATA CAA GAA AAT ACT ATT ACT AAT GAT ACT ATA GAG GAA AAA TTA GAA Ile Gln Glu Asn Thr Ile Thr Asn Asp Thr Ile Glu Glu Lys Leu Glu 885 890 895	2688
GAA CTC CAC GAA AAT GTA TTA AGT GCC GCT TTA GAA AAT ACC CAA AGT Glu Leu His Glu Asn Val Leu Ser Ala Ala Leu Glu Asn Thr Gln Ser 900 905 910	2736
GAA GAG GAA AAG AAA GAA GTA ATA GAT GTA ATT GAA GAA GTA AAA GAA Glu Glu Glu Lys Lys Glu Val Ile Asp Val Ile Glu Glu Val Lys Glu 915 920 925	2784
GAG GTC GCT AGC ACT TTA ATA GAA ACT GTC GAA CAG CCA GAA GAA AAG Glu Val Ala Thr Thr Leu Ile Glu Thr Val Glu Gln Ala Glu Glu Lys 930 935 940	2832
AGC GCA AAT ACA ATT ACC GAA ATA TTT GAA AAT TTA GAA GAA AAT GCA Ser Ala Asn Thr Ile Thr Glu Ile Phe Glu Asn Leu Glu Glu Asn Ala 945 950 955 960	2880

FIGURE 2/R

11/24

GTG GAA AGT AAT GAA AAT GTT GCA GAG AAT TTA GAG AAA TTA AAC GAA Val Glu Ser Asn Glu Asn Val Ala Glu Asn Leu Glu Lys Leu Asn Glu 965 970 975	2928
ACT GTG TTT AAT ACT GTG TTA GAT AAA GTG GAG GAA ACA GTG GAA ATT Thr Val Phe Asn Thr Val Leu Asp Lys Val Glu Glu Thr Val Glu Ile 980 985 990	2976
ACC CGA GAA AGT TTA GAA AAC AAT GAA ATG GAT AAA GCA TTT TTT AGT Ser Gly Glu Ser Leu Glu Asn Asn Glu Met Asp Lys Ala Phe Phe Ser 995 1000 1005	3024
GAA ATA TTT GAT AAT GTG AAA GGA ATA CAA GAA AAT TTA TTA ACA GGT Glu Ile Phe Asp Asn Val Lys Gly Ile Gln Glu Asn Leu Leu Thr Gly 1010 1015 1020	3072
ATG TTT CGA AGT ATA GAA ACC AGT ATA GTG ATG CAA TCA GAA GAA AAG Met Phe Arg Ser Ile Glu Thr Ser Ile Val Ile Gln Ser Glu Glu Lys 1025 1030 1035 1040	3120
GTT GAT TTG AAT GAA AAT GTG GTT AGT TCG ATT TTA GAT AAT ATA GAA Val Asp Leu Asn Glu Asn Val Val Ser Ser Ile Leu Asp Asn Ile Glu 1045 1050 1055	3168
AAT ATG AAA GAA GGT TTA TTA AAT AAA TTA GAA AAT ATT TCA AGT ACT Asn Met Lys Glu Gly Leu Leu Asn Lys Leu Glu Asn Ile Ser Ser Thr 1060 1065 1070	3216
GAA GGT GTT CAA GAA ACT GTG ACT GAA CAT GTG GAA CAA AAT GTG TAT Glu Gly Val Gln Glu Thr Val Thr Glu His Val Glu Gln Asn Val Tyr 1075 1080 1085	3264
GTG GAT GTT CAT GTT CCT GCT ATG AAA GAT CAA TTT TTA GGA ATA TTA Val Asp Val Asp Val Pro Ala Met Lys Asp Gln Phe Leu Gly Ile Leu 1090 1095 1100	3312
AAT GAG GCA GCA GCG TTC AAA GAA ATG TTT TTT AAT TTG GAA GAT GTG Asn Glu Ala Gly Gly Leu Lys Glu Met Phe Phe Asn Leu Glu Asp Val 1105 1110 1115 1120	3360
TTT AAA AGT GAA AGT CAT GTG ATT ACT GTG GAA GAA ATT AAG CAT GAA Phe Lys Ser Glu Ser Asp Val Ile Thr Val Glu Glu Ile Lys Asp Glu 1125 1130 1135	3408
CGG GTT CAA AAA GAG GTG GAA AAA GAA ACT GTT AGT ATT ATT GAA GAA Pro Val Gln Lys Glu Val Glu Lys Glu Thr Val Ser Ile Ile Glu Glu 1140 1145 1150	3456
ATG GAA GAA AAT ATT GTG GAT GTG TTA GAG GAA GAA AAA GAA GAT TTA Met Glu Glu Asn Ile Val Asp Val Leu Glu Glu Glu Lys Glu Asp Leu 1155 1160 1165	3504

FIGURE 2/F

12/24

ACA GAC AAG ATG ATA GAT GCA GTA GAA GAA TCC ATA GAA ATA TCT TCA Thr Asp Lys Met Ile Asp Ala Val Glu Glu Ser Ile Glu Ile Ser Ser 1170 1175 1180	3552
GAT TCT AAA GAA GAA ACT GAA TCT ATT AAA GAT AAA GAA AAA CAT GTT Asp Ser Lys Glu Glu Thr Glu Ser Ile Lys Asp Lys Glu Lys Asp Val 1185 1190 1195 1200	3600
TCA CTA GTT GTT GAA GAA GTT CAA GAC AAT GAT ATG GAT GAA AGT GTT Ser Leu Val Val Glu Glu Val Gln Asp Asn Asp Met Asp Glu Ser Val 1205 1210 1215	3648
GAG AAA GTT TTA GAA TTC AAA AAT ATG GAA GAG GAG TTA ATG AAG GAT Glu Lys Val Leu Glu Leu Lys Asn Met Glu Glu Glu Leu Met Lys Asp 1220 1225 1230	3696
GCT GTT GAA ATA AAT GAC ATT ACT AGC AAA CTT ATT GAA GAA ACT CAA Ala Val Glu Ile Asn Asp Ile Thr Ser Lys Leu Ile Glu Glu Thr Gln 1235 1240 1245	3744
GAG TTA AAT GAA GTA GAA GCA GAT TTA ATA AAA GAT ATG GAA AAA TTA Glu Leu Asn Glu Val Glu Ala Asp Leu Ile Lys Asp Met Glu Lys Leu 1250 1255 1260	3792
AAA GAA TTA GAA AAA CCA TTA TCA GAA GAT TCT AAA GAA ATA ATA GAT Lys Glu Leu Glu Lys Ala Leu Ser Glu Asp Ser Lys Glu Ile Ile Asp 1265 1270 1275 1280	3840
GCA AAA GAT GAT ACA TTA CAA AAA GTT ATT GAA GAG GAA CAT GAT ATA Ala Lys Asp Asp Thr Leu Glu Lys Val Ile Glu Glu Glu His Asp Ile 1285 1290 1295	3888
ACG ACG ACG TTC GAT GAA GTT GTA GAA TTA AAA GAT GTC GAA GAA GAC Thr Thr Thr Leu Asp Glu Val Val Glu Leu Lys Asp Val Glu Glu Asp 1300 1305 1310	3936
AAG ATC GAA AAA GTA TCT GAT TTA AAA GAT CTT GAA GAA GAT ATA TTA Lys Ile Glu Lys Val Ser Asp Leu Lys Asp Leu Glu Glu Asp Ile Leu 1315 1320 1325	3984
AAA GAA GTA AAA GAA ATC AAA GAA CTT GAA AGT GAA ATT TTA GAA GAT Lys Glu Val Lys Glu Ile Lys Glu Leu Glu Ser Glu Ile Leu Glu Asp 1330 1335 1340	4032
TAT AAA GAA TTA AAA ACT ATT GAA ACA GAT ATT TTA GAA GAG AAA AAA Tyr Lys Glu Leu Lys Thr Ile Glu Thr Asp Ile Leu Glu Glu Lys Lys 1345 1350 1355 1360	4080
GAA ATA GAA AAA GAT CAT TTT GAA AAA TTC GAA GAA GAA GCT GAA GAA Glu Ile Glu Lys Asp His Phe Glu Lys Phe Glu Glu Glu Ala Glu Glu 1365 1370 1375	4128

FIGURE 2/G

ATA AAA GAT CTT GAA GCA GAT ATA TTA AAA GAA GTA TCT TCA TTA GAA Ile Lys Asp Leu Glu Ala Asp Ile Leu Lys Glu Val Ser Ser Leu Glu 1380 1385 1390	4176
GTT GAA GAA GAA AAA AAA TTA GAA GAA GTA CAC GAA TTA AAA GAA GAG Val Glu Glu Glu Lys Lys Leu Glu Glu Val His Glu Leu Lys Glu Glu 1395 1400 1405	4224
GTA GAA CAT ATA ATA AGT GGT GAT GCG CAT ATA AAA GGT TTG GAA GAA Val Glu His Ile Ile Ser Gly Asp Ala His Ile Lys Gly Leu Glu Glu 1410 1415 1420	4272
GAT GAT TTA GAA GAA GTA GAT GAT TTA AAA GCA AGT ATA TTA GAC ATG Asp Asp Leu Glu Glu Val Asp Asp Leu Lys Gly Ser Ile Leu Asp Met 1425 1430 1435 1440	4320
TTA AAC GGA GAT ATG GAA TTA GGG CAT ATG GAT AAG GAA AGT TTA GAA Leu Lys Gly Asp Met Glu Leu Gly Asp Met Asp Lys Glu Ser Leu Glu 1445 1450 1455	4368
GAT GTA ACA ACA AAA CTT GGA GAA AGA GTT GAA TCC TTA AAA GAT GTT Asp Val Thr Thr Lys Leu Gly Glu Arg Val Glu Ser Leu Lys Asp Val 1460 1465 1470	4416
TTA TGT AGT CCA TTA GGC ATG GAT GAA GAA CAA ATG AAA ACA AGA AAA Leu Ser Ser Ala Leu Gly Met Asp Glu Glu Gln Met Lys Thr Arg Lys 1475 1480 1485	4464
AAA GCT CAA AGA CTT AAG TTG GAA GAA GTA TTA TTA AAA GAA GAG GTT Lys Ala Gln Arg Pro Lys Leu Glu Glu Val Leu Leu Lys Glu Glu Val 1490 1495 1500	4512
AAA GAA GAA CCA AAG AAA AAA ATA ACA AAA AAG AAA GTA AGC TTT GAT Lys Glu Glu Pro Lys Lys Lys Ile Thr Lys Lys Lys Val Arg Phe Asp 1505 1510 1515 1520	4560
ATT AAG GAT AAG GAA CCA AAA GAT GAA ATA GTA GAA GTT GAA ATG AAA Ile Lys Asp Lys Glu Pro Lys Asp Glu Ile Val Glu Val Glu Met Lys 1525 1530 1535	4608
GAT GAA GAT ATA GAA GAA GAT GTA GAA GAA CAT ATA GAA GAA GAT ATA Asp Glu Asp Ile Glu Glu Asp Val Glu Glu Asp Ile Glu Glu Asp Ile 1540 1545 1550	4656
GAA GAA GAT AAA GTT GAA GAT ATA GAT GAA GAT ATA GAT GAA GAT ATA Glu Glu Asp Lys Val Glu Asp Ile Asp Glu Asp Ile Asp Glu Asp Ile 1555 1560 1565	4704
GGT CAA GAC AAA GAT GAA GTT ATA GAT TTA ATA GTC CAA AAA GAG AAA Gly Glu Asp Lys Asp Glu Val Ile Asp Leu Ile Val Gln Lys Glu Lys 1570 1575 1580	4752

FIGURE 2/H

CGC ATT GAA AAC GTT AAA GCG AAA AAG AAA AAA TTA GAA AAA AAA GTT Arg Ile Glu Lys Val Lys Ala Lys Lys Lys Lys Leu Glu Lys Lys Val 1585 1590 1595 1600	4800
GAA GAA GGT GTT ACT GGT CTT AAA AAA CAC GTA CAC GAA GTA ATG AAA Glu Glu Gly Val Ser Gly Leu Lys Lys His Val Asp Glu Val Met Lys 1605 1610 1615	4848
TAT GTT CAA AAA ATT GAT AAA GAA GTT GAT AAA GAA GTA TGT AAA GCT Tyr Val Gln Lys Ile Asp Lys Glu Val Asp Lys Glu Val Ser Lys Ala 1620 1625 1630	4896
TTA GAA TCA AAA AAT GAT GTT ACT AAT GTT TTA AAA CAA AAT CAA GAT Leu Glu Ser Lys Asn Asp Val Thr Asn Val Leu Lys Gln Asn Gln Asp 1635 1640 1645	4944
TTT TTT AGT AAA GTT AAA AAC TTC GTA AAA AAA TAT AAA GTA TTT GCT Phe Phe Ser Lys Val Lys Asn Phe Val Lys Lys Tyr Lys Val Phe Ala 1650 1655 1660	4992
GCA CCA TTC ATA TGT GCG GTT GCA CCA TTT GCA TCA TAT GTA GTT GGC Ala Pro Phe Ile Ser Ala Val Ala Ala Phe Ala Ser Tyr Val Val Gly 1665 1670 1675 1680	5040
TTC TTT ACA TTT TGT TTA TTT TCA TCA TGT GTA ACA ATA GCT TCT TCA Phe Phe Thr Phe Ser Leu Phe Ser Ser Cys Val Thr Ile Ala Ser Ser 1685 1690 1695	5088
ACT TAC TTA TTA TCA AAA GTT GAC AAA ACT ATA AAT AAA AAT AAG CAG Thr Tyr Leu Leu Ser Lys Val Asp Lys Thr Ile Asn Lys Asn Lys Glu 1700 1705 1710	5136
ACA CCG TTT TAT TCA TTT GTA TTT GAT ATC TTT AAG AAT TTA AAA CAT Arg Pro Phe Tyr Ser Phe Val Phe Asp Ile Phe Lys Asn Leu Lys His 1715 1720 1725	5184
TAT TTA CAA CAA ATG AAA GAA AAA TTT AGT AAA GAA AAA AAT AAT AAT Tyr Leu Gln Gln Met Lys Glu Lys Phe Ser Lys Glu Lys Asn Asn Asn 1730 1735 1740	5232
GTA ATA GAA GTA ACA AAC AAA GGT CAG AAA AAA GGT AAT GTA CAG GTA Val Ile Glu Val Thr Asn Lys Ala Glu Lys Lys Gly Asn Val Gln Val 1745 1750 1755 1760	5280
ACA AAT AAA ACC GAG AAA ACA ACT AAA GTT GAT AAA AAT AAT AAA GTA Thr Asn Lys Thr Glu Lys Thr Thr Lys Val Asp Lys Asn Asn Lys Val 1765 1770 1775	5328
CCG AAA AAA AGA AGA ACG CAA AAA TCA AAA TAA Pro Lys Lys Arg Arg Thr Gln Lys Ser Lys *	5361
1780 1785	

FIGURE 2/1

15/24

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1891 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..1891

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

T ACA TTA ACT GAA AGT GTA CAT GAT AAT AAA AAT TTA GAA GAA GCC	46
Thr Leu Thr Glu Ser Val Asp Asp Asn Lys Asn Leu Glu Glu Ala	
1 5 10 15	
GAA GAT ATA AAG CAA AAT ATC TTA TTA ACT AAT ATA GAA GAA GCA AAA	94
Glu Asp Ile Lys Glu Asn Ile Leu Leu Ser Asn Ile Glu Glu Pro Lys	
20 25 30	
GAA AAT ATT ATT GAC AAT TTA TTA AAT AAT ATT GGA CAA AAT TCA GAA	142
Glu Asn Ile Ile Asp Asn Leu Leu Asn Asn Ile Gly Gln Asn Ser Glu	
35 40 45	
AAA CAA GAA AGT GTA TCA GAA AAT GTA CAA GTC AGT GAT GAA CTT TTT	190
Lys Gln Glu Ser Val Ser Glu Asn Val Gln Val Ser Asp Glu Leu Phe	
50 55 60	
AAT GAA TTA TTA AAT ACT GTA GAT GTT AAT GGA CAA GTA AAA GAA AAT	238
Asn Glu Leu Leu Asn Ser Val Asp Val Asn Gly Glu Val Lys Glu Asn	
65 70 75	
ATT TTG CAG GAA AGT CAA GTT AAT GAC GAT ATT TTT AAT AGT TTA GTA	286
Ile Leu Glu Glu Ser Gln Val Asn Asp Asp Ile Phe Asn Ser Leu Val	
80 85 90 95	
AAA AGT GTT CAA CAA CAA CAA CAA CAC AAT GTT CAA GAA AAA GTT GAA	334
Lys Ser Val Gln Gln Glu Gln Gln His Asn Val Glu Glu Lys Val Glu	
100 105 110	
GAA AGT GTA GAA GAA AAT GAC CAA GAA ACT GTA GAA GAA AAT GTA GAA	382
Glu Ser Val Glu Glu Asn Asp Glu Glu Ser Val Glu Glu Asn Val Glu	
115 120 125	
GAA AAT GTA GAA CAA AAT GAC GAC GGA AGT GTA GCC TCA AGT GTT GAA	430
Glu Asn Val Glu Glu Asn Asp Asp Gly Ser Val Ala Ser Ser Val Glu	
130 135 140	

FIGURE 3/A

GAA AGT ATA GCT TCA AGT GTT GAT GAA AGT ATA GAT TCA AGT ATT GAA Glu Ser Ile Ala Ser Ser Val Asp Glu Ser Ile Asp Ser Ser Ile Glu 145 150 155	478
GAA AAT GTA GCT CCA ACT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA Glu Asn Val Ala Pro Thr Val Glu Glu Ile Val Ala Pro Thr Val Glu 160 165 170 175	526
GAA ATT GTA GCT CCA AGT GTT GTA GAA AGT GTG GCT CCA AGT GTT GAA Glu Ile Val Ala Pro Ser Val Val Glu Ser Val Ala Pro Ser Val Glu 180 185 190	574
GAA AGT GTA GCT CCA AGT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA Glu Ser Val Ala Pro Ser Val Glu Glu Ser Val Ala Glu Asn Val Glu 195 200 205	622
GAA AGT GTA GCT GAA AAT GTT GAA GAA ATC GTA GCT CCA AGT GTT GAA Glu Ser Val Ala Glu Asn Val Glu Glu Ile Val Ala Pro Ser Val Glu 210 215 220	670
GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu 225 230 235	718
GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu 240 245 250 255	766
GAA AGT GTA GCT GAA AAT GTT GAA GAA ATC GTA GCT CCA ACT GTT GAA Glu Ser Val Ala Glu Asn Val Glu Glu Ile Val Ala Pro Thr Val Glu 260 265 270	814
GAA AGT GTA GCT CCA ACT GTT GAA GAA ATT GTA GCT CCA ACT GTT GAA Glu Ser Val Ala Pro Thr Val Glu Glu Ile Val Ala Pro Thr Val Glu 275 280 285	862
GAA AGT GTA GCT CCA ACT GTT GAA GAA ATT GTA GTT CCA AGT GTT GAA Glu Ser Val Ala Pro Thr Val Glu Glu Ile Val Val Pro Ser Val Glu 290 295 300	910
GAA AGT GTA GCT CCA AGT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA Glu Ser Val Ala Pro Ser Val Glu Glu Ser Val Ala Glu Asn Val Glu 305 310 315	958
GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu 320 325 330 335	1006
GAA AGT GTA GCT GAA AAT GTT GAA GAA AGT GTA GCT GAA AAT GTT GAA Glu Ser Val Ala Glu Asn Val Glu Glu Ser Val Ala Glu Asn Val Glu 340 345 350	1054

FIGURE 3/B

CAA	ATC	GTA	GCT	CCA	AGT	GTT	GAA	GAA	ATC	GTA	GCT	CCA	ACT	GTT	GAA	1102
Glu	Ile	Val	Ala	Pro	Ser	Val	Glu	Glu	Ile	Val	Ala	Pro	Thr	Val	Glu	
			355					360					365			
GAA	AGT	CTT	GCT	GAA	AAC	GTT	GCA	ACA	AAT	TTA	TCA	GAC	AAT	GTT	TTA	1150
Glu	Ser	Val	Ala	Glu	Asn	Val	Ala	Thr	Asn	Leu	Ser	Asp	Asn	Leu	Leu	
		370					375					380				
AGT	AAT	TTA	TTA	GGT	GGT	ATC	GAA	ACT	CAC	GAA	ATA	AAG	GAC	AGT	ATA	1198
Ser	Asn	Leu	Leu	Gly	Gly	Ile	Glu	Thr	Glu	Glu	Ile	Lys	Asp	Ser	Ile	
		385				390					395					
TTA	AAT	GAG	ATA	GAA	GAA	GTA	AAA	GAA	AAT	GTA	GTC	ACC	ACA	ATA	GTA	1246
Leu	Asn	Glu	Ile	Glu	Glu	Val	Lys	Glu	Asn	Val	Val	Thr	Thr	Ile	Leu	
400						405				410					415	
GAA	AAA	GTA	GAA	GAA	ACT	ACA	GCT	GAA	AGT	GTA	ACT	ACT	TTT	AGT	AAT	1294
Glu	Lys	Val	Glu	Glu	Thr	Thr	Ala	Glu	Ser	Val	Thr	Thr	Phe	Ser	Asn	
				420					425					430		
ATA	TTA	GAG	GAG	ATA	CAA	GAA	AAT	ACT	ATT	ACT	AAT	GAT	ACT	ATA	GAG	1342
Ile	Leu	Glu	Glu	Ile	Gln	Glu	Asn	Thr	Ile	Thr	Asn	Asp	Thr	Ile	Glu	
			435					440					445			
GAA	AAA	TTA	GAA	GAA	CTC	CAC	CAA	AAT	GTA	TTA	AGT	CCC	GCT	TTA	GAA	1390
Glu	Lys	Leu	Glu	Glu	Leu	His	Glu	Asn	Val	Leu	Ser	Ala	Ala	Leu	Glu	
		450					455					460				
AAT	ACC	CAA	AGT	GAA	GAG	GAA	AAG	AAA	GAA	GTA	ATA	GAT	GTA	ATT	GAA	1438
Asn	Thr	Gln	Ser	Glu	Glu	Glu	Lys	Lys	Glu	Val	Ile	Asp	Val	Ile	Glu	
		465				470					475					
GAA	GTA	AAA	GAA	GAG	GTC	GCT	ACC	ACT	TTA	ATA	GAA	ACT	GTC	GAA	CAG	1486
Glu	Val	Lys	Glu	Glu	Val	Ala	Thr	Thr	Leu	Ile	Glu	Thr	Val	Glu	Gln	
480					485					490					495	
GCA	GAA	GAA	GAG	ACC	CAA	AGT	ACA	ATT	ACG	GAA	ATA	TTT	GAA	AAT	TTA	1534
Ala	Glu	Glu	Glu	Ser	Glu	Ser	Thr	Ile	Thr	Glu	Ile	Phe	Glu	Asn	Leu	
				500					505					510		
GAA	GAA	AAT	GCA	GTA	GAA	AGT	AAT	GAA	AAA	GTT	GCA	GAG	AAT	TTA	GAG	1582
Glu	Glu	Asn	Ala	Val	Glu	Ser	Asn	Glu	Lys	Val	Ala	Glu	Asn	Leu	Glu	
			515					520					525			
AAA	TTA	AAC	GAA	ACT	GTA	TTT	AAT	ACT	GTA	TTA	GAT	AAA	GTA	GAG	GAA	1630
Lys	Leu	Asn	Glu	Thr	Val	Phe	Asn	Thr	Val	Leu	Asp	Lys	Val	Glu	Glu	
		530					535					540				
ACA	GTA	GAA	ATT	AGC	GCA	GAA	AGT	TTA	GAA	AAC	AAT	GAA	ATG	GAT	AAA	1678
Thr	Val	Glu	Ile	Ser	Gly	Glu	Ser	Leu	Glu	Asn	Asn	Glu	Met	Asp	Lys	
		545				550					555					

FIGURE 3/C

GCA	TTT	TTT	AGT	GAA	ATA	TTT	GAT	AAT	GTA	AAA	CGA	ATA	CAA	GAA	AAT	1726
Ala	Phe	Phe	Ser	Glu	Ile	Phe	Asp	Asn	Val	Lys	Gly	Ile	Gln	Glu	Asn	
560					565					570					575	
TTA	TTA	ACA	GGT	ATG	TTT	CGA	AGT	ATA	GAA	AGC	AGT	ATA	GTA	ATC	CAA	1774
Leu	Leu	Thr	Gly	Met	Phe	Arg	Ser	Ile	Glu	Thr	Ser	Ile	Val	Ile	Gln	
			580						585					590		
TCA	GAA	GAA	AAG	GTT	GAT	TTC	AAT	GAA	AAT	CTG	GTT	AGT	TCG	ATT	TTA	1822
Ser	Glu	Glu	Lys	Val	Asp	Leu	Asn	Glu	Asn	Val	Val	Ser	Ser	Ile	Leu	
			595					600					605			
GAT	AAT	ATA	GAA	AAT	ATC	AAA	GAA	CGT	TTA	TTA	AAT	AAA	TTA	GAA	AAT	1870
Asp	Asn	Ile	Glu	Asn	Met	Lys	Glu	Gly	Leu	Leu	Asn	Lys	Leu	Glu	Asn	
		610					615					620				
ATT	TCA	AGT	ACT	GAA	GGC	GAA										1891
Ile	Ser	Ser	Thr	Glu	Gly	Glu										
	625					630										

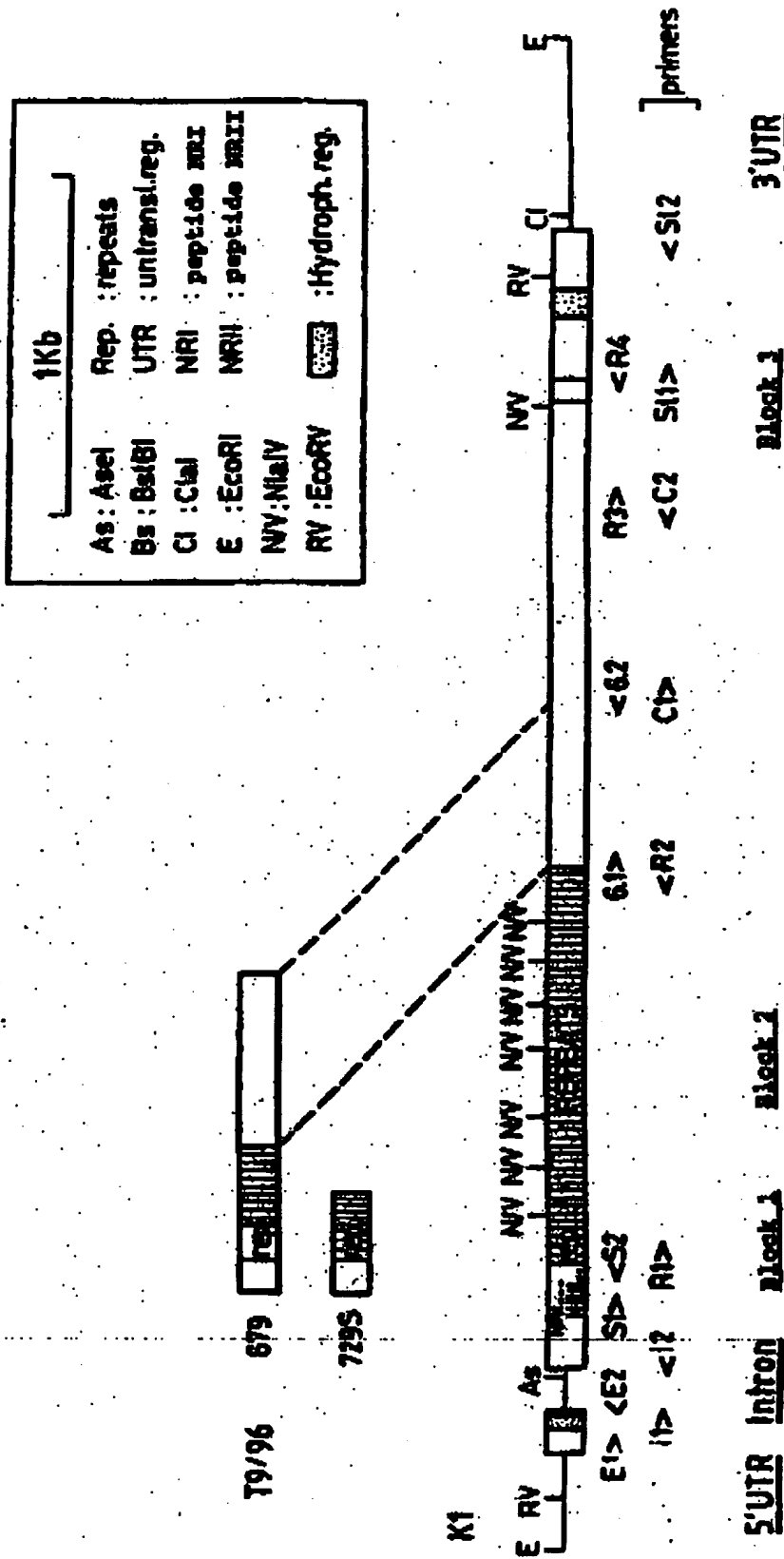


FIGURE 4

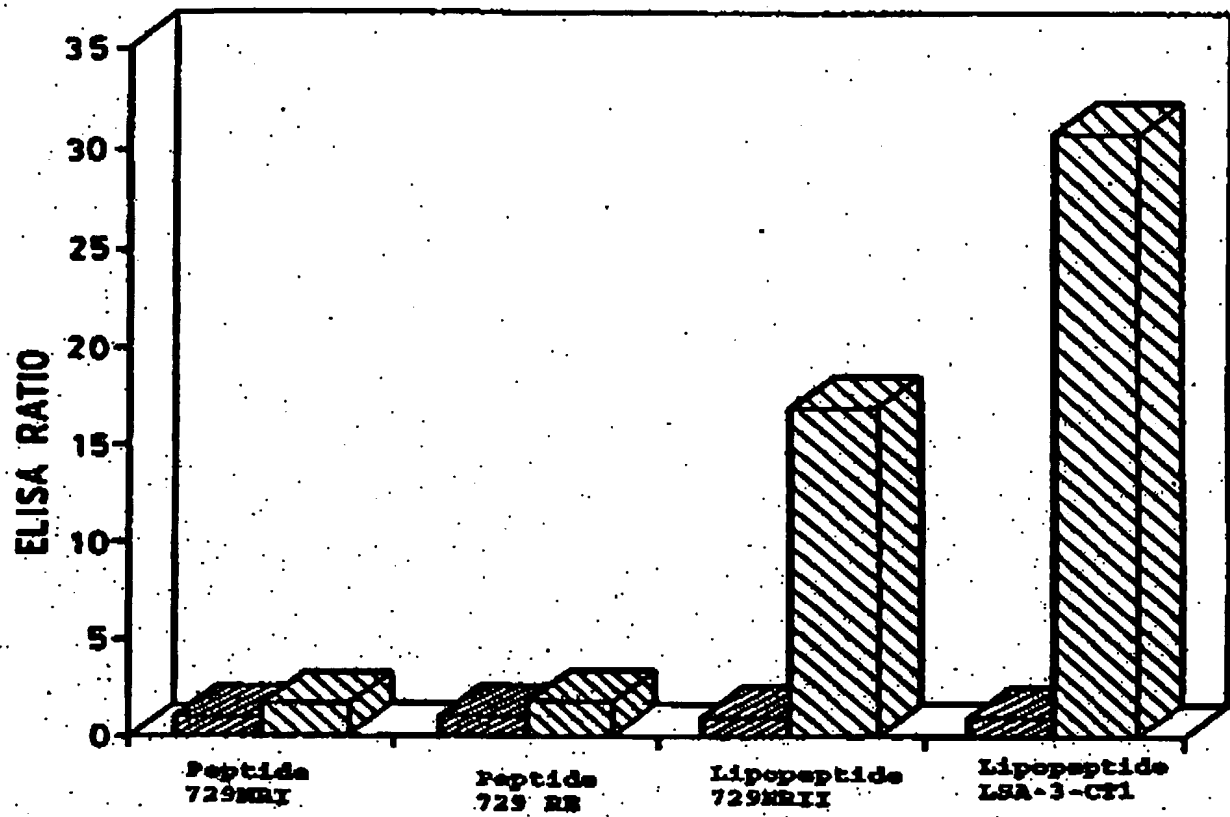


FIGURE 5

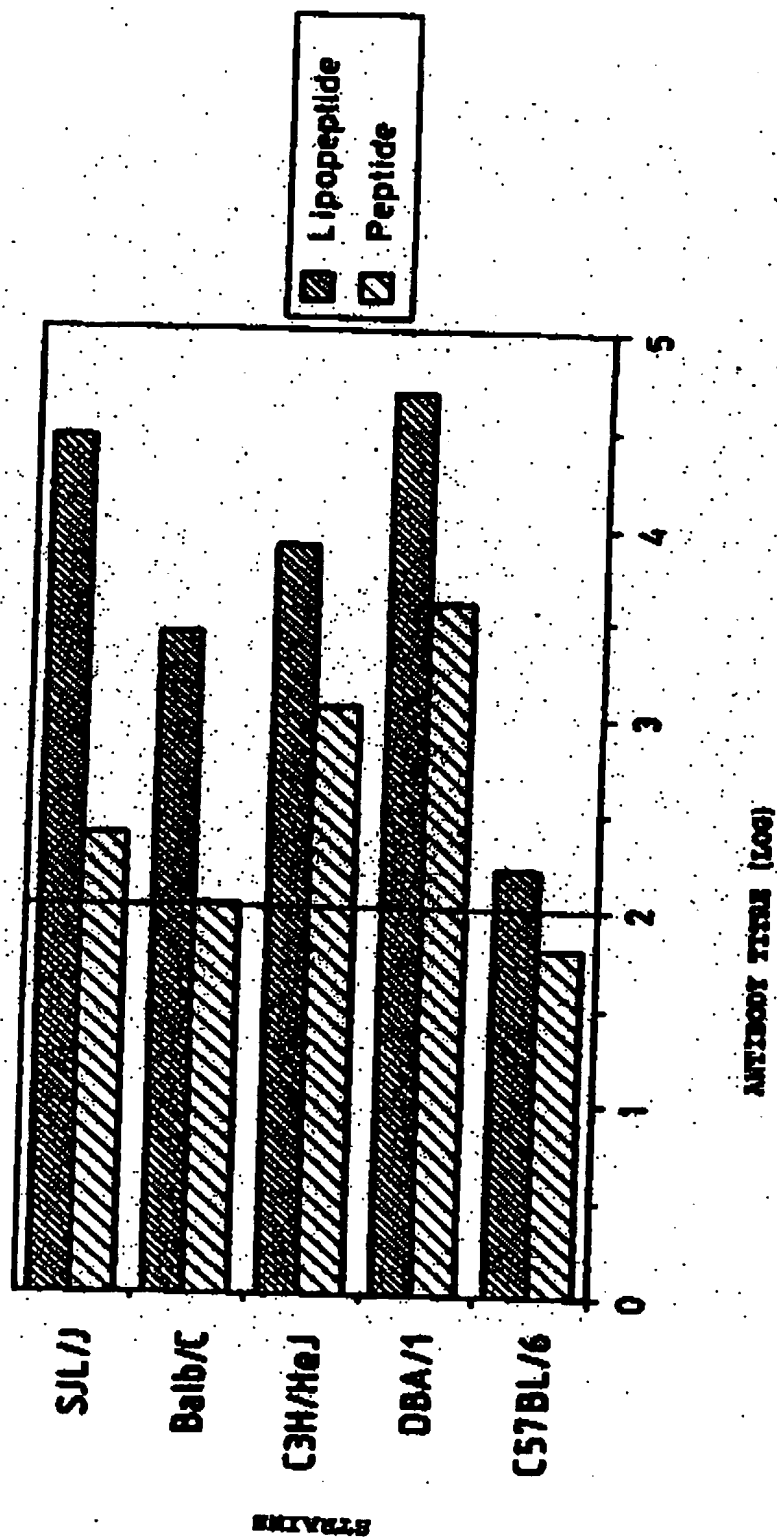


FIGURE 6

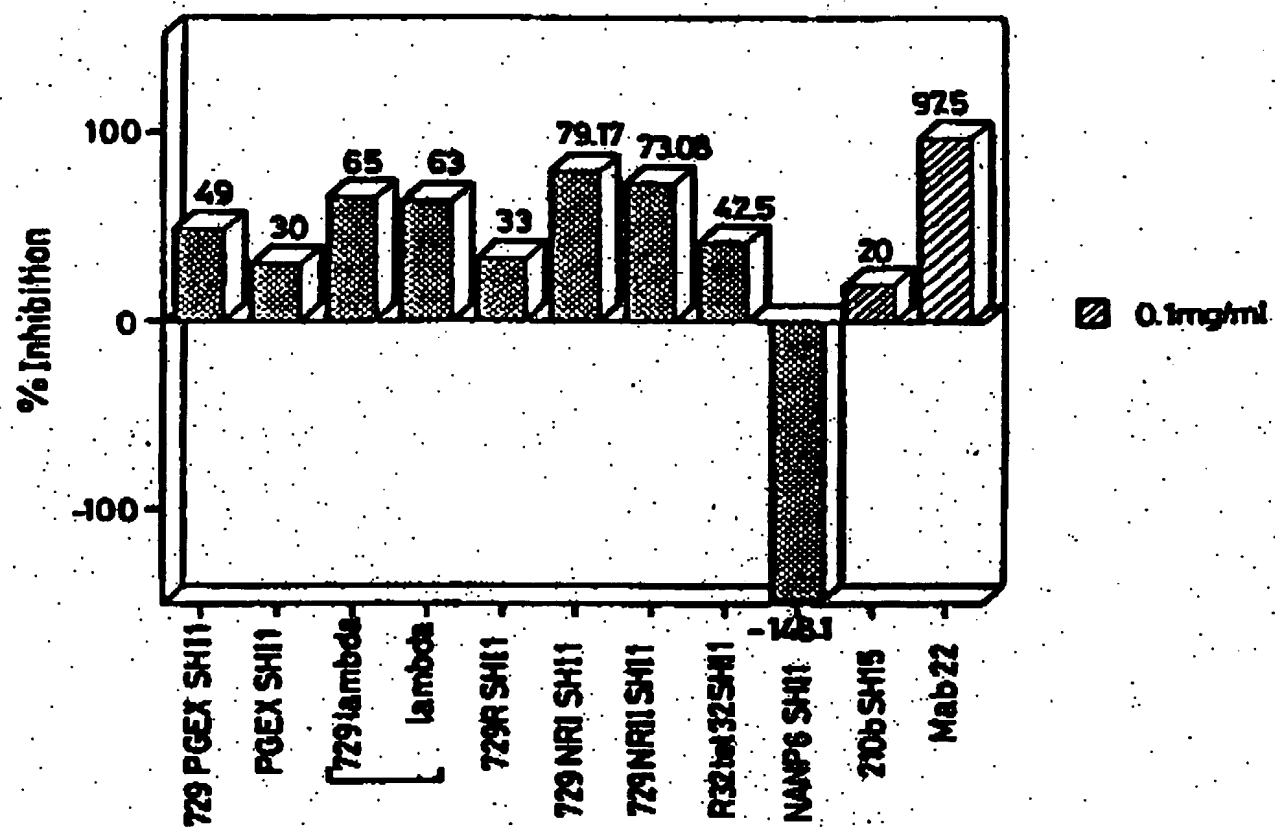


FIGURE 7

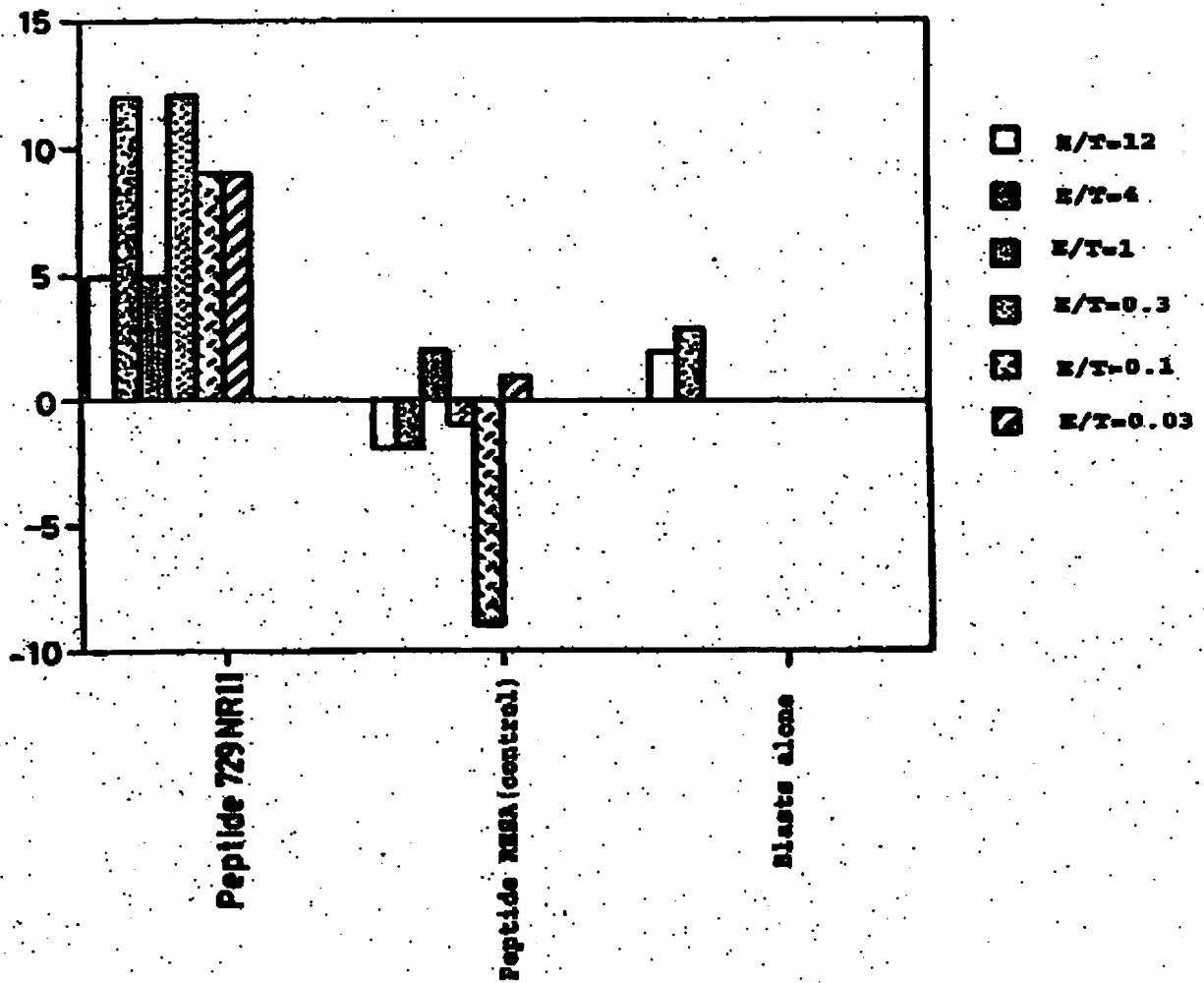


FIGURE 8

▨ Before immunization

□ After immunisation

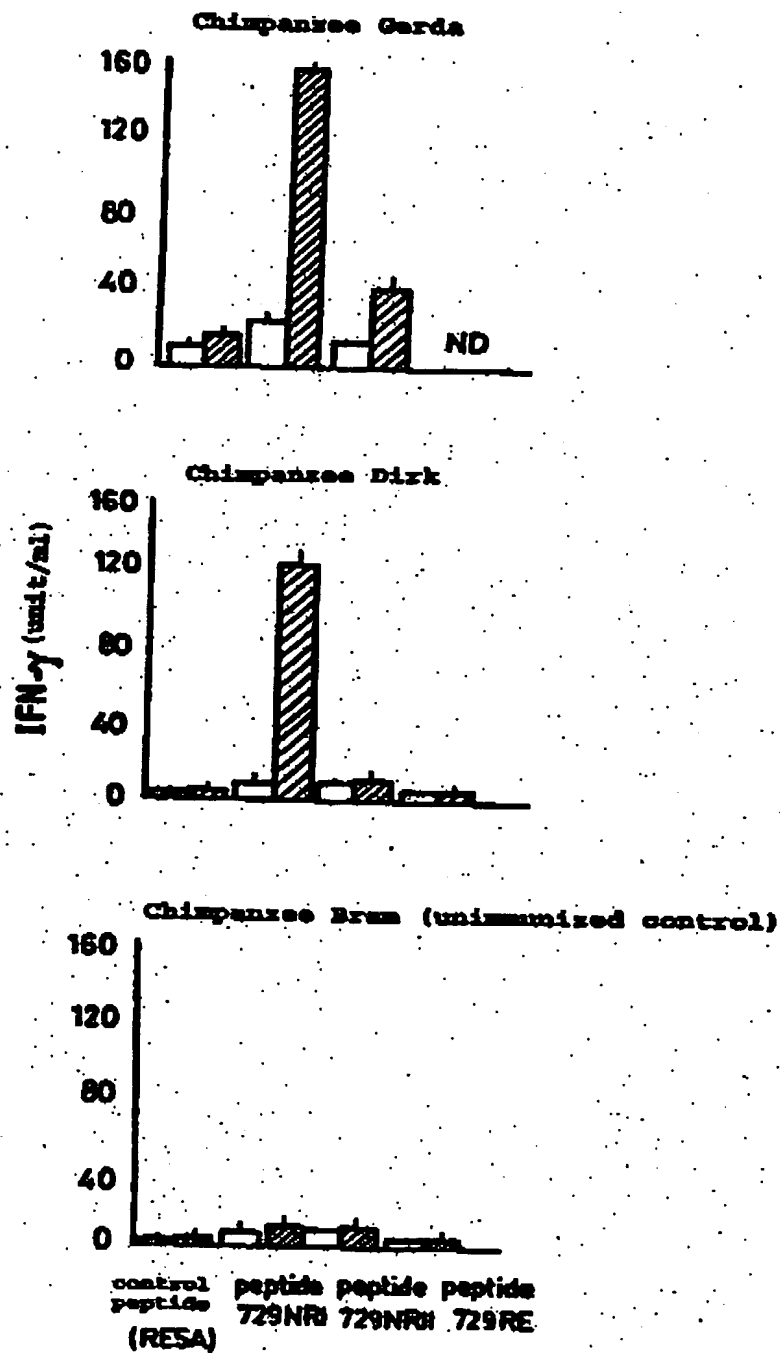


FIGURE 9

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